ESTIMATION OF SERUM URIC ACID CONCENTRATION IN CORONARY HEART DISEASE PATIENTS

By

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APPROVAL

This thesis, by **Muhammad Anwar** is accepted in its present form by the Department of Biological Sciences, Quaid-i-Azam University, Islamabad, as satisfying the thesis requirements for the degree of Master of Philosophy in Biochemistry / Molecular biology.

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Dedicated

To

MY FAMILY

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AMP	Adenosine mono-phosphate
ATP	Adenosine tri-phosphate
BMI	Body mass index
CHD	Coronary heart disease
Chl	Cholesterol
ETT	Exercise Stress Test
GMP	Guanosine mono-phosphate
HDL	High Density Lipoprotein
IDDM	Insulin dependent diabetes mellitus
IHD	Ischiemic heart disease
IMP	Inosine mono-phosphate
LDL	Low Density Lipoprotein
MI	Myocardial Infarction
NIDDM	Non-insulin dependent diabetes mellitus
POD	Peroxidase
SD	Standard Deviation
SUA	Serum Uric Acid
Tg	Triglyceride
Veg	Vegetarian
XMP	Xanthosine mono-phosphate

ABSTRACT

Serum uric acid (SUA) level was estimated in fifty healthy and fifty disease patients to find out its correlation with coronary heart disease (CHD) events. Among CHD groups these patients were diagnosed by myocardial infarction, angiography, thallium scan, exercise stress test (ETT) either singularly or in various combinations. A positive correlation has been found between elevated SUA level and CHD. The study has revealed that patients with CHD has raised SUA which was statistically significant (0.036). Mean values of SUA concentration for healthy and coronary heart disease patients were found to be 298.28 ± 94.58 and 333.88 ± 105.80, μmol/L respectively.

A correlation of serum uric acid was also found with age, body mass index (BMI) and diet of CHD patients. With different age groups viz. 35-39, 40-44, 45-49 and 50-54 years, SUA level was found to be 303.12 ± 114.53, 349.00 ± 113.28, 337.35 ± 92.03 and 378.00 ± 115.61 μmol/L in CHD patients which is higher respectively than SUA level of healthy individuals in these groups. With different ranges of BMI viz. 18-20, 21-23, 24-26 and 27-29 of healthy and CHD patients, SUA level increased with an increase in BMI in both the groups. Persons with CHD (28% fatty) showed an increase in serum uric acid level than normal healthy subjects. Higher serum uric acid has been found in fatty (overweight) and old age peoples. Such information determine certain metabolites can be proven valuable in finding out the actual causes of CHD.

INTRODUCTION
AND
REVIEW OF LITERATURE

CHAPTER # 1

INTRODUCTION

Serum uric acid (SUA), a product of purine metabolism, is degraded in most mammals by the hepatic enzyme, urate oxidase (uricase), to allantoine, which is freely excreted in the urine (Wu *et al.*, 1992). SUA levels also vary significantly within humans as the result of factors that increased generation (such as high purine or protein diet, alcohol consumption, condition with high cell turnover or enzymatic defects in purine metabolism) or decrease excretion. A reduction in glomerular filtration rate (GFR) increases SUA, although a significant compensatory increase in gastrointestinal excretion occurs (Vaziri *et al.*, 1995).

The kidney excretes serum uric acid as a waste product. The kidney excretes two-third of the uric acid produced daily; the remaining one-third is excreted in the stool. The exact level of SUA that is considered pathological is controversial. In recent years, it has been recognize that the normal ranges of serum uric acid are quite wide. Because of this wide range, SUA levels show day-to-day and seasonal variations in the same persons. Urine uric acid levels may also be used to evaluate gout or determine over secretion of uric acid.

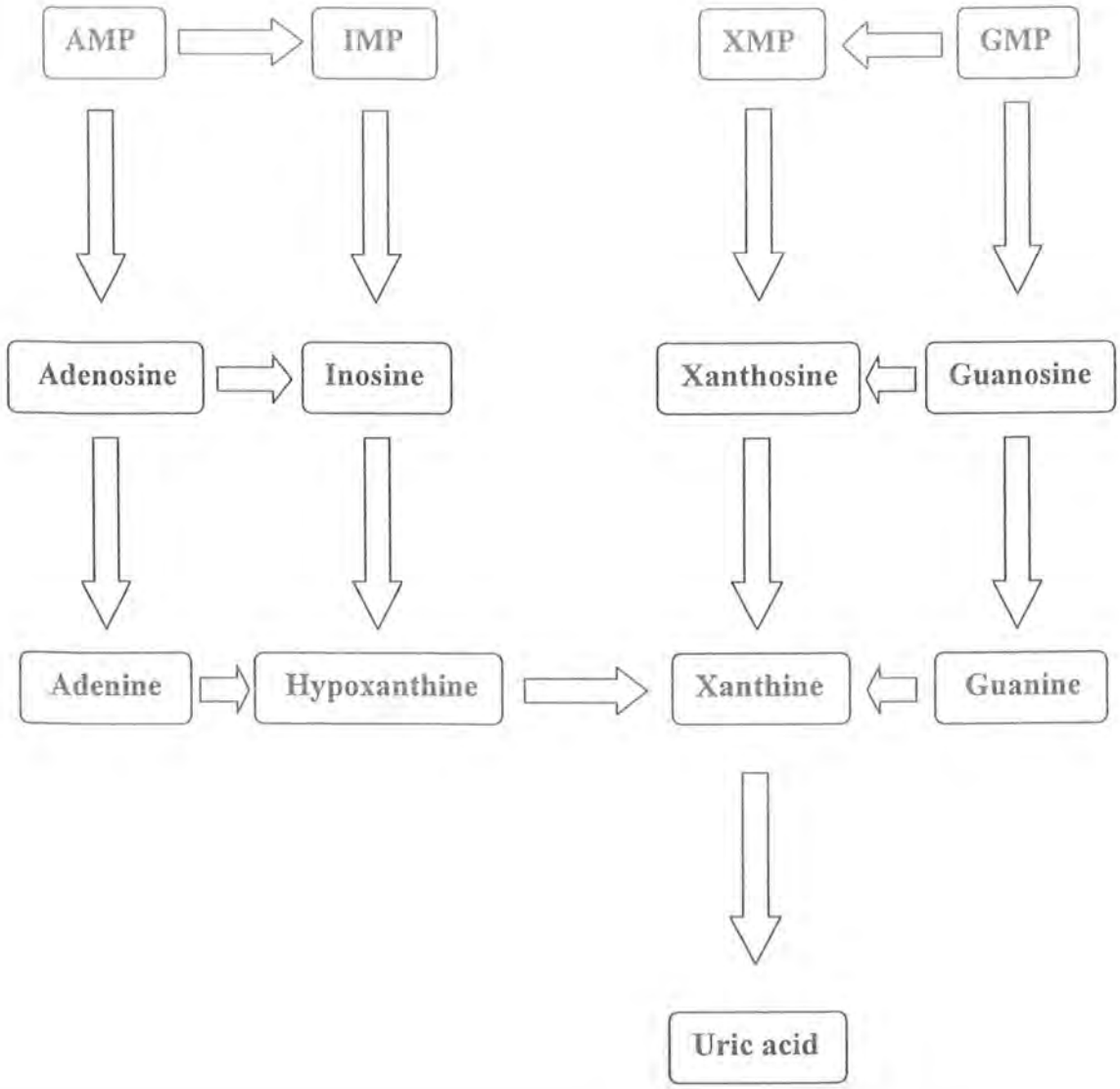

Fig. 1.1 Flow sheet of uric acid biosynthesis

Reference values of serum uric acid are,

- Adult male : 2.5-7.0 mg/dl 148 – 416 μ mol/L
- Adult females : 2.5-6.0 mg/dl 148 – 357 μ mol/L

➢ **Children (ages 10-18)**

- Males : 3.6-5.5 mg/dl 214 – 327 μ mol/L
- Females : 3.6-4.0 mg/dl 214 – 237 μ mol/L

➢ **Elderly**

- Male older than 40 : 2.0-8.5 mg/dl 118 – 505 μ mol/L
- Female older than 40 : 2.0-8.0 mg/dl 118 – 475 μ mol/L

The normal range for urinary uric acid is between 250 - 750 mg (1.5 - 4.5μ mol/L) over a 24-hour period. An elevated blood uric acid level also known as hyperuricemia, is seen in

- Gout
- Renal disease and renal failure
- Alcoholism
- Dehydration
- Leukemia and lymphoma
- Starvation
- Metabolic acidosis

An overproduction of serum uric acid occurs when there is excessive cell breakdown and catabolism of nucleonic acid such as seen in gout. Excessive production and destruction of cells, as may occur in leukemia or during cancer therapy, or problems with SUA excretion due to renal failure (Leal-Pinto *et al.*, 1999).

1.1 GOUT

Serum uric acid causes problems because human does not posses the enzyme to digest it to a soluble form. When SUA precipitates, it can cause kidney stones or gout. Gout is problem where SUA crystals deposit in the joints, causing a painful inflammatory response.

1.2 STONE FORMATION

Like any stone, uric acid stones form when too much uric acid is present in the urine to remain dissolved. Uric acid stones form quickly as there are no known inhibitors in human urine to cope with fluctuation in out put. A short period of dehydration in a susceptible individual is enough to begin stone formation. A sudden uric acid load from food can also precipitate a new stone. This means that what you eat and drink directly affects your chance of developing stone.

1.3 URIC ACID FROM FOOD

Uric acid solubility in urine is dependent on the pH, or acidity of the urine. At a pH of 7.0, urine can dissolve thousand times the amount of uric acid than at pH 5.0. Most

people who form frequent uric acid stones have acidic urine. Urine become acidic in response to diet. Fifty percent of uric acid in the body comes from food.

1.4 CAUSE OF HYPERURICEMIA

Hyperuricemia has several causes. In diseases such as leukemia, increased cellular breakdown provides high levels of purine and increase purine turn over. Production of SUA as an end product of this metabolism increases. Several diuretics (thiazides) cause uric acid to increase in serum, either due to dehydration as more body water is lost or because of the blockage of the tubular secretion of uric acid. Patients with diabetic ketoacidosis, renal failure or rapid weight loss have impaired tubular secretion of uric acid, leading to elevated serum level (Calbrath, 1992).

1.5 RISK FACTOR FOR CORONARY HEART DISEASE

Risk factor is a trait that predicts the risk of development of clinically significant disease within the population (Brounwald, 1971). In some case it may involved in the causation of the disease, however it require a proven epidemiological association that is statistically valid. The risk factor concept has been developed from the famous Framingham studies which completed fourty years follow-up and the data was presented by Dr. W.B. Kannel at the American College of Cardiology (Gordon *et al.*, 1982; Kannel and Schatzkin, 1983).

The risk factor concept has been extremely useful because it permits one to asses the importance of not only previously mentioned risk factor but also of genetic trait in

given individual such as family history of premature disease. Thus a risk factor may be defined broadly as "any habit or trait that can be used to predict an individual probability of developing that disease". A risk factor also defined may be causative agent but is not necessary one, a more limited and specific definition is that "a risk factor is a causative agent or condition that can be used to predict an individual probability of developing disease". So according to definition there are some independent predictors of risk for individuals within a population of the incidence of atherosclerosis. The association between these risk factors and incidence of coronary heart disease has been established by several prospective epidemiological studies in the United State and Europe. These studies demonstrate a consistent association among characteristics observed at one point in time in apparently healthy individual with the subsequent incidence of coronary heart disease in those individuals. So as a result of these associations, each characteristic has been termed a risk factor for CHD.

1.6 CORONARY HEART DISEASE

Coronary heart disease is the genetic designation for a group of closely related syndromes resulting from an imbalance between the supply and demand of heart for oxygenated blood. Depending on the rate of development of the arterial narrowing and its ultimate severity, four coronary syndromes may result:

1 Angina pectoris, of which there are three variants, the most threatening being unstable angina.

2 Myocardial infarction, the most important form of CHD.

3 Chronic CHD or coronary cardiomyopathy.

4 Sudden cardiac death, which may be super imposed on any of the proceeding three conditions (Heger et al., 1994; Cotran and Robins, 1989; Gyton, 1971).

Coronary heart disease is a multi factorial disease with its pathogenic roots in the first and second decade of life (Goldberg, 1992). There is a little doubt that coronary heart disease for its obvious clinical heterogeneity, belongs to the class of multi factorial disorder. The important clues are to be found in Mendelian conditions that confer an increase risk of premature heart disease. Most of the autosomal recessive conditions are relatively rare. The fact that many, but not all, monogenetic conditions with predisposition to coronary heart disease suggest that genes effecting the function and quantity of plasma lipids and lipoprotein may be seen as candidate genes (Brock, 1993).

1.7 FACTORS ASSOCIATED WITH CORONARY HEART DISEASE

1.7.1 Family History of Coronary Heart Disease Patients & Genetics

Studies of the family history of coronary heart disease suggest that family history is an important predictor for the risk of subsequent coronary heart disease (Chadha, 1998; Silberg *et al.*, 1998). Particularly in men, with a risk approximately one and one-half to two times greater for those with a parental history of coronary heart disease (Goldberg, 1992). Framingham study subjects with a brother who developed coronary heart disease experienced a doubling in coronary disease risk and increased risk was not attributable to

shared factors (Snowden *et al.*, 1982). Coronary heart disease is often multi factorial in origin, resulting from the interplay of genetic and environmental factors (Keating and Sanguinetti, 1996).

1.7.2 AGE

There is a marked increase in the risk of coronary heart disease with increasing age. During the first fourteen years of follow-up in the Framingham study, among those found to be initially free from coronary heart disease, every eighth man 40-44 years of age at the time of study entry had developed some form of coronary heart disease; the percentage of men developing coronary heart disease increased with age to approximately every sixth man aged 45-49 years at entry, every fifth man aged 50-54 years at entry, and every fourth man 55 years of the age or older at entry (Castelli and Leaf, 1985). In the population-based Worcester Heart Attack study, the risk of dying during the acute hospital phase for those with an initial acute myocardial infarction, were 1.1, 2.9 and 7.5 times greater for patients 55-64 years, 65-74 years and 75 years and older, respectively, relative to the referent group of patient less than 55 years of age (Golberg *et al.*, 1989).

1.7.3 SEX

Coronary heart disease is a major health concern for both men and women. In the United State, males typically exhibit higher age. Specific incidence rates of coronary heart disease than women throughout life, though the difference in attack rates of coronary heart disease tends to narrow after the menopause, with coronary heart disease

becoming a major cause of morbidity and mortality among women beyond their mid-to late 50s (Kannel *et al.*, 1987). Women suffering acute myocardial infarction are older than men (an average 8-10 years older) and exhibit higher crude in hospital case-fatality rates (Fieback *et al.*, 1990; Robinson *et al.*, 1988).

1.7.4 SMOKING

Cigarette smoking appears to be the most important risk factor for CHD in countries where the incidence of CHD is higher (Kannel, 1978). The rate in smokers has generally been 2-3 times that of non-smokers and is dose related, with no evidence that non-inhalation or the use of filter cigarette offer any protection. Recent evidence suggests that passive smokers may also be at high risk. Cigarette smoking not only promotes premature coronary atherosclerosis but also has important effects on coagulation (Kannel, 1987) and the sympathetic nervous system (Winniford *et al.*, 1986). All of these may contribute to the increase risk of coronary heart disease. Ex-smokers are at high risk of acute coronary heart disease for at least 15 years (Robison *et al.*, 1989) and upto 20 years after giving up smoking than people who have never smoked (Cook *et al.*, 1986). Cigarette smokers have a higher risk of CHD than non-smokers do (Kannel, 1981).

1.7.5 HYPERTENSION

Hypertension is a global problem, incidence varies from 10-20 % in developed and developing countries. The exact incidence in our country is unknown. When a person is said to have hypertension or high blood pressure, it is generally mean that his or her means arterial pressure is greater than the upper of accepted normality. In sever

hypertension, the mean arterial pressure often rises to as high as 150-170 mm Hg, with diastolic pressure as high as 130-150 mm Hg and systolic arterial pressure some time as great as 210 mm Hg.

Elevated blood pressure either systolic or diastolic is predictive of an increased risk of developing coronary heart disease (Flack and Wiist, 1991). In general blood pressure increases with age, especially during the first 50-60 years of life. Because of the increase in systolic blood pressure, the pulse pressure tends to increase (Weber *et al.*, 1989).

1.7.6 DIABETES MELLITUS

Diabetics are not only at increased risk of death from diabetes but they are also at increased risk of death from CHD, stroke and other circulatory diseases (Kleinman *et al.*, 1988; Kannel *et al.*, 1986). Diabetes mellitus is a clinical syndrome characterized by hyperglycemia, due to deficiency or diminished effectiveness of insulin (Fayyaz *et al.*, 1988), and is often accompanied by the presence of glucose in the urine, from which the name of this condition is derived (Guyton, 1971). Diabetes is a serious metabolic disease and affects the metabolism of coronary heart disease, proteins, fats, water and electrolytes (Malik and Bukhari, 1995). Most of the pathology of diabetes mellitus can be attributed to one of the following three major effects of insulin lack.

1. Decrease utilization of glucose by body cell with a resulting increase in blood glucose concentration to as high as 300-500 mg/dl.

2. Markedly increased metabolization of fats from the fat storage areas, causing abnormal fat metabolism as well as deposition of lipids in vascular walls to cause atherosclerosis, and

3. Depletion of protein in the tissues of body.

The two major types of diabetes mellitus are insulin dependent and non insulin dependent diabetes mellitus (Lipson, 1984). Insulin dependent diabetes mellitus affects approximately 0.3 % of individuals aged under 20 years. The insulin deficiency of IDDM is based on auto-immune destruction of pancreatic islets β-cells (Simpson *et al.*, 1984).

Non insulin dependent diabetes mellitus is associated with an increased CHD risk. NIDDM is characterized by both insulin resistance and β-cells dysfunction (Reaven, 1988; Polonsky *et al.*, 1988). Insulin resistance is defined as a state in which given amount of insulin does not produce the expected biological response i.e. the transport of glucose into the body cells. They may lead to the series of increasingly severe metabolic abnormalities, including the development of type II diabetes mellitus (Saud *et al.*, 1991).

1.7.7 OBESITY

It is recommended that clinicians first classify patients by body mass index (BMI), calculated as weight in kg divided by the square of height in meters, with overweight defined as a BMI of 25.0 – 29.9 kg/m^2 and obesity as a BMI at least 30 kg/m^2 (NIH, 1998). Obesity has been considered as one of the factors in the genesis of essential hypertension (Chiang *et al.*, 1969). Morbid obesity is a significant medical

problem in Pakistan, affecting approximately 45% of men and 65 % of women (Qadri *et al.*, 1996). Obesity is associated with hypertension, stroke, coronary heart disease and diabetes mellitus (Bray, 1985; Van, 1979). In men at least height appears to be independent transmissible risk factor for coronary heart disease (Kee *et al.*, 1997).

1.7.8 HYPERLIPIDEMIA

Framingham study shows a beneficial effect of increase levels of HDL cholesterol with decrease risk of coronary heart disease in both men and women (Castelli and Leaf, 1985). Low HDL cholesterol is a risk factor for coronary heart disease and is usually associated with increase triglyceride level (Chadha, 1998). Serum Tg level now recognized as an independent risk factor for CHD (Castelli, 1986). The LDL cholesterol level decreases, the relative risk also decreases. So LDL is often included in the assessment of the risk of coronary heart disease.

High serum uric acid level is a risk factor in coronary heart disease. (Galvan *et al.*, 1995). With ischemia, ATP is degraded to adenine and xanthine, and there is also increased generation of xanthene oxidase. The increased availability of substrate (xanthine) and enzyme (xanthene oxidase) result in increased uric acid formation. The finding that ischemia resulted in an increased serum uric acid level (Many *et al.*, 1996). Elevated SUA is associated with subjects at CHD risk may count for hyperuricemia that predict the development of coronary heart disease in the general population in subjects with hypertension and with pre-existing CHD (Tuttle *et al.* 2002). SUA is not an independent risk factor for CHD after controlling for these other risk factors.

Hyperuricemia is therefore considered unless associated with gout or kidney stone (Duffy *et al.* 1981). SUA have a pathogenic role in hypertension and CHD (Richard *et al.* 2003). Most epidemiological evidence suggests a significant, graded, independent and specific association between the level of serum uric acid and CHD morbidity and mortality (Alderman and Michael 2002).

Serum uric acid is linked to the heart disease deaths. Peoples with high levels of uric acid in their blood had an increased risk of dying from heart disease. The presence of elevated SUA identifies a sign of greater risk of CHD mortality. That effect is greater in women than in men and considerably greater in African Americans than in whites. Among men, the risk was 77% higher with the highest levels compared with those with the lowest levels. In both men and women, the relationship between SUA and heart-related deaths was only present in people aged 45 and older. People aged 45 to 54 who has high levels of SUA appeared to have the highest risk. Increased SUA levels are linked to obesity, distorted cholesterol levels and high blood pressure (Mercola 2000).

Serum uric acid is not an independent risk factor for coronary heart disease. There is much controversy concerning the role of uric acid as an independent risk factor in the development of coronary heart disease because serum uric acid is related to many of the established etiologic risk factors for CHD. This review finds little support for an independent causal role for serum uric acid in the development of coronary heart disease (Wannamethee 2001). Alderman (2001) also discussed serum uric acid as a CHD risk factor for heart disease. Elevated SUA is frequently found in-patients with kidney and

CHD. Reduced renal clearance of urate resulted in elevated serum uric acid level (Culleton 2001).

Increase in serum uric acid in subjects with CHD might therefore reflect a compensatory mechanism to counter the oxidative stress that occurs in these conditions. However this does not readily explain higher SUA level in patient with CHD are generally associated with worse out come (Ames *et al.*, 1981). Hyperuricemia causes hypertension, internal vascular disease, renal disease and vascular inflammation that also provide the long-sought pathogenic mechanism by which SUA could cause CHD in humans (Klein, 1973). There is relationship between raised serum uric acid and subsequent CHD events i.e. mortality, myocardial infarction, stroke. Hyperuricemia was significantly associated with a twofold increased risk of both myocardial infarction and stroke incidence in men. However, hyperuricemia was significantly related to a double risk of all mortality and stroke onset (Longo et al. 1999).

It remains unclear that serum uric acid is a risk factor for progression in subjects with established renal disease. Although experimental studies suggest serum uric acid acts as risk factor for progression (Tabayashi *et al.*, 1991). Perez *et al.* (2000) reported an improvement in renal function with the lowering of serum uric acid in gouty subjects, other have not been able to confirm these findings.

Serum uric acid also stimulates the production of cytokines from leukocytes and chemokines from vascular smooth muscle cells. This suggests a potential role for uric

acid or for xanthene oxidase in mediating the systemic inflammatory response that is linked to CHD events. Finally these studies may provide an insight into that serum uric acid is not always found to be an independent risk factor for CHD events. A recent sub analysis showed that the cardio-production was lost in those treated patients in whom serum uric acid levels increased (Franse *et al.* 2000).

Fang and Alderman (2000) discussed that SUA may not always be a risk factor for CHD due to the reason that beneficial antioxidant actions of serum uric acid may partially counter its potential detrimental effects. It is of interest that almost all studies examining the relation of SUA level with CHD events showed a J-shaped curve. Although it is possible that increased risk at higher level reflects the role of serum uric acid in inducing vascular disease and hypertension.

Aging also effects on SUA level. Serum uric acid is related not only to an increased risk of gout, but also to be an increased risk of coronary heart disease. Serum uric acid levels in men and women increased with advancing age, despite changes in drinking and in the body mass index (BMI) (Kuzuya *et al.*, 2002).

Association between the SUA and coronary heart disease is gender dependent. The upper serum uric acid in the women group was associated with higher coronary heart disease severity than the lower serum uric acid. There is also association between age and CHD as serum uric acid and other variables did not have significant independent association with CHD (Lu *et al.* 2002).

Recent evidence supported a role for SUA as a true CHD risk factor. Studies need to be performed in humans to prove or disproved this possibility before lowering serum uric acid is routinely recommended (Richard *et al.*, 2003). Serum uric acid found to play a role as risk factor in coronary heart disease. The present study was therefore conducted to establish the correlation of serum uric acid as a risk factor in coronary heart disease within Pakistani population.

MATERIALS AND METHODS

CHAPTER # 2

MATERIALS AND METHODS

Serum samples of 100 subjects were drawn and analyzed for uric acid. The uric acid concentrations were estimated in healthy as well as cardiovascular disease patients. The effect of uric acid as a risk factor was analyzed in cardiovascular diseases. Association between age, sex, diet and BMI with uric acid was observed in healthy and cardiovascular disease patients. The concentrations of uric acid in serum samples were determined with the help of enzymatic colorimetric method. The complete protocol adopted was as under.

2.1 COLLECTION OF SAMPLES

Serum samples of 100 subjects were drawn out of 170 because of exclusion criteria (Table 2.1). Out of 100, fifty subjects were healthy and the remaining were coronary artery diseased patients. All the subjects were male and between the age of 35-54 years. The duration of disease and treatment was nearly the same for all fifty coronary artery disease patients. Written consent was taken from each person before collecting the serum samples. The personal history, age, sex, diet, height, body weight and salary of subjects were recorded from the written consent. The BMI was calculated as,

$$BMI = \frac{\text{Weight (kg)}}{\text{Height (m}^2\text{)}}$$

Table 2.1 Inclusion and Exclusion criteria used for the selection of serum samples

Inclusion Criteria	Exclusion Criteria
Established CHD	Diabetes Mellitus
Age between 35-55	Renal Disease
-	Obesity (BMI>30)
-	Hyperuricemia / Gout
-	Hypertension
-	Hyperlipedemia
-	Smoker

2.2 BIOCHEMICAL ANALYSIS OF SERUM SAMPLES

Serum samples collected from all subjects were analyzed for serum uric acid, cholesterol; Tg, HDL, and LDL cholesterol using prepack enzymatic kit. All analysis was made automatically using blood auto analyzer.

2.2.1 ESTIMATION OF SERUM URIC ACID

Uric acid was estimated in the serum samples by enzymatic kit method (Uricase-PAP).

I) Principal

Uric acid is oxidized by uricase to allantoine and hydrogen peroxide, which under the influence of peroxidase (POD), oxidizes DCPS and 4-AP to form a red quinoneimine compound.

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoine} + CO_2 + 2H_2O_2$$

$$2H_2O_2 + 4AP + DCPS \xrightarrow{\text{POD}} \text{Quinoneimine} + 4\ H_2O$$

The quantity of this red quinoneimine formed is proportional to the uric acid concentration.

II) REAGENTS

The reagents used for the determination of uric acid are presented in Table 2.2.

Table 2.2 Reagents used for the determination of serum uric acid

Reagent 1	Phosphate pH 7.4	50 mmol/L
Buffer Solution	2-4 DCPS	4 mmol/L
Reagent 2	Uricase	60 U/L
Enzyme	Peroxidase	660 U/L
	Ascorbate-Oxidase	200 U/L
	4-Aminophenazone	1 mmol/L
Standard	Uric acid solution	6 mg/dl

III) PREPARATION AND STABILITY

Dissolve the contents of one bottle R.2, to the contents of one bottle buffer solution R.1. This working reagent is stable four weeks at 2-8 °C or 10 days at room temperature, when stored in a dark bottle.

IV) PROCEDURE

For enzymatic colorimetric test for serum uric acid estimation, auto-analyzer was adjusted to zero against reagent blank at wavelength 520 nm (490-550 nm) at room temperature. The cuvette of 1cm light path was used for the analysis of each sample. The auto-analyzer gave automatically final results. Each time cuvette was rinsed with distilled water and auto-analyzer was adjusted to zero before each sample reading. Assay protocol for the determination of uric acid level is given in Table 2.3.

V) CALCULATION

Quantity of uric acid was calculated as,

$$\text{Uric acid (mg/dl)} = \frac{\text{Ext. sample}}{\text{Ext. standard}} \times \text{Conc. Standard (mg/dl)}$$

mg/dl x 59,485 = µmol/L

Conc. Standard = 6.0 mg/dl

VI) LINEARITY

This method is linear upto 25 mg/dl (1487 µmol/L). If the uric acid concentration is greater than 25 mg/dl in the serum, dilute the sample 1:2 with saline solution and repeat the determination, and multiply by 2.

Table 2.3 Assay protocol of uric acid in serum samples

	Blank	Standard	Sample
Standard	-	25µl	-
Sample	-	-	25µl
Reagent	1.0 ml	1.0 ml	1.0 ml
Mix, incubate 10 minute at room temperature. The colour is stable for 30 minute.			

G) REFERENCE VALUES

Reference values of serum uric acid for men and women are 3.4-7.0 mg/dL (202-416 µmol/L) and 2.5-6.0 mg/dL (148-357 µmol/L), respectively. Uric acid in serum is stable for 3-5 days when stored at 2-8 °C.

2.2.2 ESTIMATION OF LIPID PROFILE

A) CHOLESTEROL

I) Principal

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestene-4-en-one} + H_2O_2$$

$$H_2O_2 + \text{4-Aminophenazone} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinonimine} + H_2O$$

II) Reagents

Phosphate buffer (pH 6.5)	100 mmol/L
4-Aminophenazone	0.4 mmol/L
Phenol	5 mmol/L
Peroxidase	1250 U/L
Cholesterol esterase	300 U/L
Cholesterol oxidase	300 U/L

III) Standard

Cholesterol sol.	200 mg/dL

IV) Method

For enzymatic colorimetric test for cholesterol estimation, auto analyzer was set at wave length 546 nm, adjusted to zero against reagent blank at 37 °C. 10 µl of serum and 1.0 ml of reagent were mixed and incubated for five minutes at 37 °C and concentration was measured. The auto analyzer automatically gave final results of cholesterol concentration.

B) TRIGLYCERIDE (Tg)

I) Principal

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + \text{Fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerokinase}} \text{Glycerol-3-Phosphate} + \text{ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{Glycerol phosphate oxidase}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-Aminophenazone} \xrightarrow{\text{Peroxidase}} \text{Quinonimine} + H_2O + HCl + \text{4-chloroPhenol}$$

II) Reagents

Reagent / R-1 (Buffer)

PIPES buffer (pH 7.5)	50 mmol/L
4-Chlorophenol	5 mmol/L
Magnesium ions	5 mmol/L
ATP	1 mmol/L
Lipase	≥ 1.0 U/ml
Peroxidase	≥ 0.5 U/ml
Glycerol kinase	≥ 0.4 U/ml
Sodium azide	0.05 %

Reagent / R-2 (Enzyme Reagent)

4-Amiophenazone	≥ 0.13 mmol/L
Glycerol-3-phosphate oxidase	≥ 1.5 U/ml
Sodium azide	0.095 %

III) Method

For enzymatic colorimetric test for triglyceride estimation, auto analyzer was set at wave length 546 nm, adjusted to zero against reagent blank at 37 °C. 10 µl of serum and 1.0 ml of reagent were mixed and incubated for five minutes at 37 °C and concentration was measured. The auto analyzer automatically gave final results of triglyceride concentration.

C) HDL CHOLESTEROL

I) Reagents

R-1 Precipitation Reagent (250 ml)

Phosphotungstic acid	1.14 mmol/L
Magnesium chloride	8.6 mmol/L

R-2 Reaction solution for cholesterol determination

Phosphate buffer (pH 6.5)	100 mmol/L
4-Aminophenazone	0.4 mmol/L
Phenol	5 mmol/L
Peroxidase	1250 U/L
Cholesterol esterase	300 U/L
Cholesterol oxidase	300 U/L

R-3	Standard
Cholesterol standard solution	50 mg/dl

II) Method

1ml of precipitant reagent and 500 µl of serum were pipetted into a test tube and mixed well; incubated for 10 minutes at room temperature. The sample was centrifuged at 10000 g for 2 minutes. After this pure supernatant was separated and used for HDL cholesterol determination.

For enzymatic colorimetric test for HDL cholesterol estimation, auto analyzer was set at wave length 546 nm, adjusted to zero against reagent blank at 37 °C. 100 µl of HDL supernatant and 1.0 ml of reagent were mixed and incubated for five minutes at 37 °C and concentration was measured. The auto analyzer automatically gave final results of HDL cholesterol concentration.

D) LDL CHOLESTEROL

LDL concentration was calculated from the total cholesterol concentration (TC) by using the following formula,

LDL-Cholesterol = Total Cholesterol – HDL-Cholesterol – Tg

STATISTICAL ANALYSIS

The statistical analysis carried out for this study includes percentage, mean, standard error, variance, standard deviation and t-test by using Social sciences software package (SPSS 8.0 and SPSS 10.0). The significance value was calculated by applying paired sample t-test analysis.

RESULTS

CHAPTER # 3

RESULTS

A total of 100 subjects were studied during the research work. All the subjects were male and of age between 35-55 years. There were 50 normal healthy subjects and 50 were cardiac patients of same age and sex. Normal subjects were not cardiac, hypertensive and diabetic patients while others are pure coronary heart disease patients. The coronary heart disease patients were diagnosed by cardiologist with the help of angiography, thallium scan, ETT or history of myocardial infarction (Fig. 3.1). which shows that among CHD patients, the incidence of myocardial infarction (MI) was 16 %, angiography 36%, ETT / thallium 16%, thallium 8%, ETT / angiography 2%, MI / angiography 12%, MI / thallium 2% and ETT 8% while among healthy group ETT was found to 94% and ETT / thallium was 6%.

Different characteristics including various risk factors and eating habits were also studied. A comparison was also made between dietary habits of healthy and coronary heart disease individuals. Fig. 3.2 describes among healthy individuals, 16% were fatty, 44 % vegetarian and 40 % were mixed while among coronary artery disease persons, 28% were fatty, 32% vegetarian and 40% were found to be mixed individuals

The mean ± SD values for age, body mass index (BMI), dietary habits, lipid profile and uric acid is presented in Table 3.1, whereas the data of uric acid concentration in fifty healthy and coronary heart disease patients is given in Table 3.2. Mean ± SD values of uric acid concentration for healthy and disease patients were found to be 298.28 ± 94.58 and 333.88 ± 105.80, respectively.

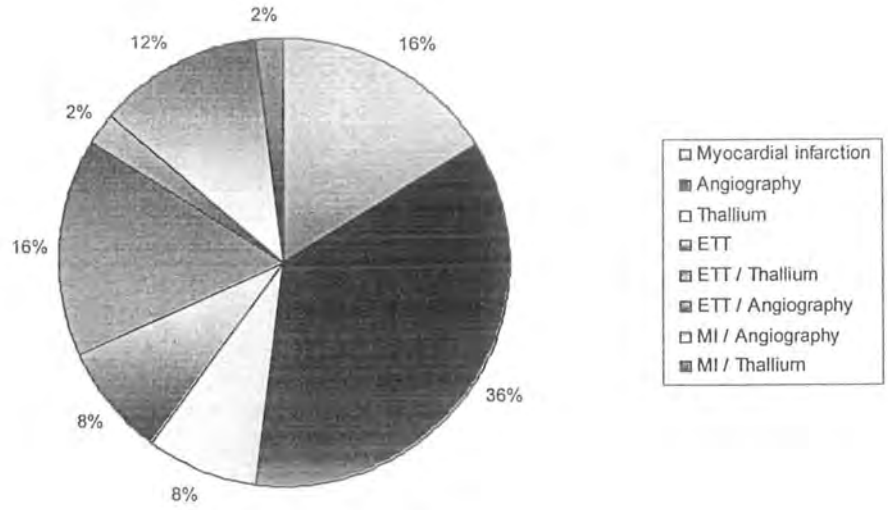

Fig. 3.1 Diagnostic criteria used for coronary heart disease patients

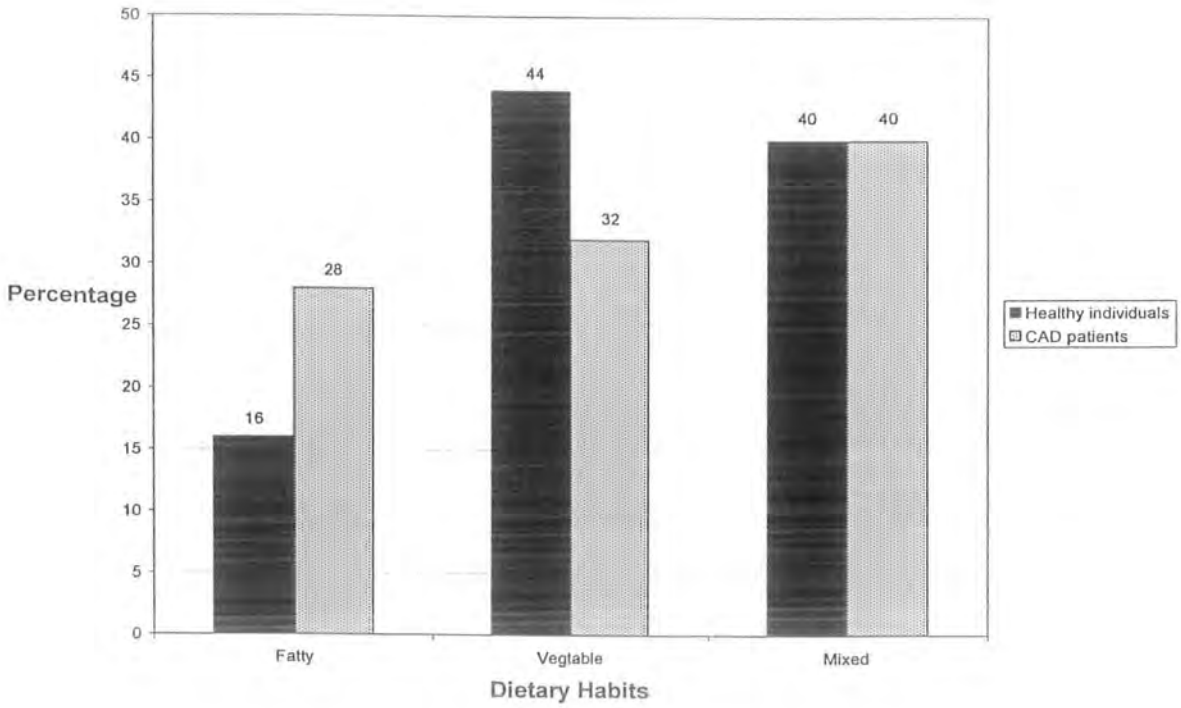

Fig. 3.2 **Percentages of different dietary habits in healthy and coronary heart disease (CHD) subjects**

Table 3.1 **Mean ± SD values of general characteristics (age, BMI, dietary habits and uric acid) for healthy and disease groups**

S. No.	Types of variables	Group I	Group II
1	Total numbers	50	50
2	Age	42.40 ± 5.38	44.26 ± 4.94
3	Sex	All male	
4	Dietary habits		
	Fatty	16 %	28 %
	Veg.	44 %	32 %
	Mixed	40 %	40 %
5	BMI (kg/m^2)	24.24 ± 2.42	24.67 ± 2.82
6	Uric acid (μmol/L)	298.28 ± 94.58	333.88 ± 105.80

Group I = Healthy group

Group II = CHD group

Table 3.2 **Uric acid concentrations (μmol/L) in healthy and coronary heart disease patients**

Number of subjects	Group I	Group II
1	285.00	420.00
2	419.00	390.00
3	373.00	381.00
4	401.00	510.00
5	201.00	159.00
6	425.00	179.00
7	199.00	277.00
8	377.00	477.00
9	253.00	377.00
10	253.00	303.00
11	177.00	219.00
12	289.00	299.00
13	199.00	271.00
14	275.00	503.00
15	501.00	301.00
16	311.00	279.00
17	301.00	427.00
18	179.00	297.00
19	299.00	333.00
20	377.00	233.00
21	333.00	275.00
22	387.00	397.00
23	155.00	211.00
24	169.00	511.00
25	195.00	218.00
26	263.00	277.00
27	423.00	339.00
28	257.00	297.00
29	305.00	419.00
30	244.00	211.00
31	371.00	399.00
32	299.00	553.00
33	421.00	277.00
34	167.00	201.00
35	250.00	499.00

Continued .....

Number of subjects	Group I	Group II
36	300.00	200.00
37	197.00	423.00
38	416.00	214.00
39	291.00	416.00
40	251.00	299.00
41	233.00	263.00
42	513.00	399.00
43	401.00	511.00
44	304.00	201.00
45	200.00	210.00
46	287.00	299.00
47	158.00	403.00
48	300.00	499.00
49	241.00	351.00
50	489.00	287.00
Mean ± SD	298.28 ± 94.58	333.88 ± 105.80

Group I = Healthy individuals

Group II = CAD patients

Significance value = 0.036

Table 3.5 **Uric acid correlation with different age groups of healthy and coronary artery disease patients**

Age groups (Years)	Uric acid concentration (μmol/L)	
	Healthy individuals	CAD individuals
35-39	268.26 ± 81.40	303.12 ± 114.53
40-44	298.30 ± 84.60	349.00 ± 113.28
45-49	313.90 ± 91.11	337.35 ± 92.03
50-54	350.00 ± 129.66	378.00 ± 115.61

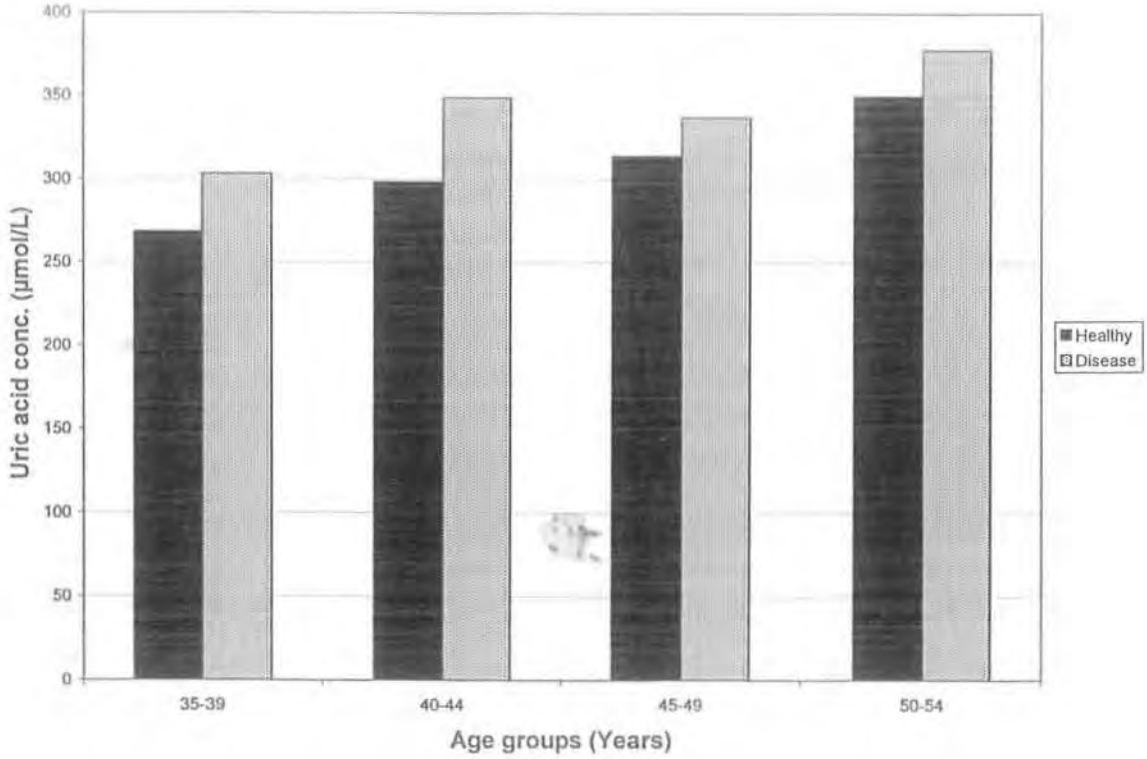

Fig. 3.3 **Effect of different age groups on serum uric acid level in healthy and coronary heart disease patients**

3.1.2 Uric acid correlation with BMI

Uric acid level was determined in four different ranges of BMI viz. 18-20, 21-23, 24-26 and 27-29 in healthy as well as coronary artery disease patients (Table 3.6; Figure 3.4).

3.1.3 Uric acid correlation with coronary artery disease (CAD)

Uric acid level was determined in coronary artery disease patients as well as healthy individuals. A correlation of SUA was found in CHD patients. Serum uric acid level was higher in CHD patients than healthy subjects (Fig. 3.3-3.4).

Table 3.6 Uric acid correlation with BMI of healthy and coronary heart disease patients

BMI	Uric acid concentration (µmol/L)	
kg/m^2	Healthy individuals	CAD individuals
18-20	278.60 ± 91.94	336.00 ± 110.91
21-23	338.27 ± 95.90	340.66 ± 115.07
24-26	261.80 ± 85.77	303.77 ± 104.87
27-29	330.66 ± 89.37	372.72 ± 90.00

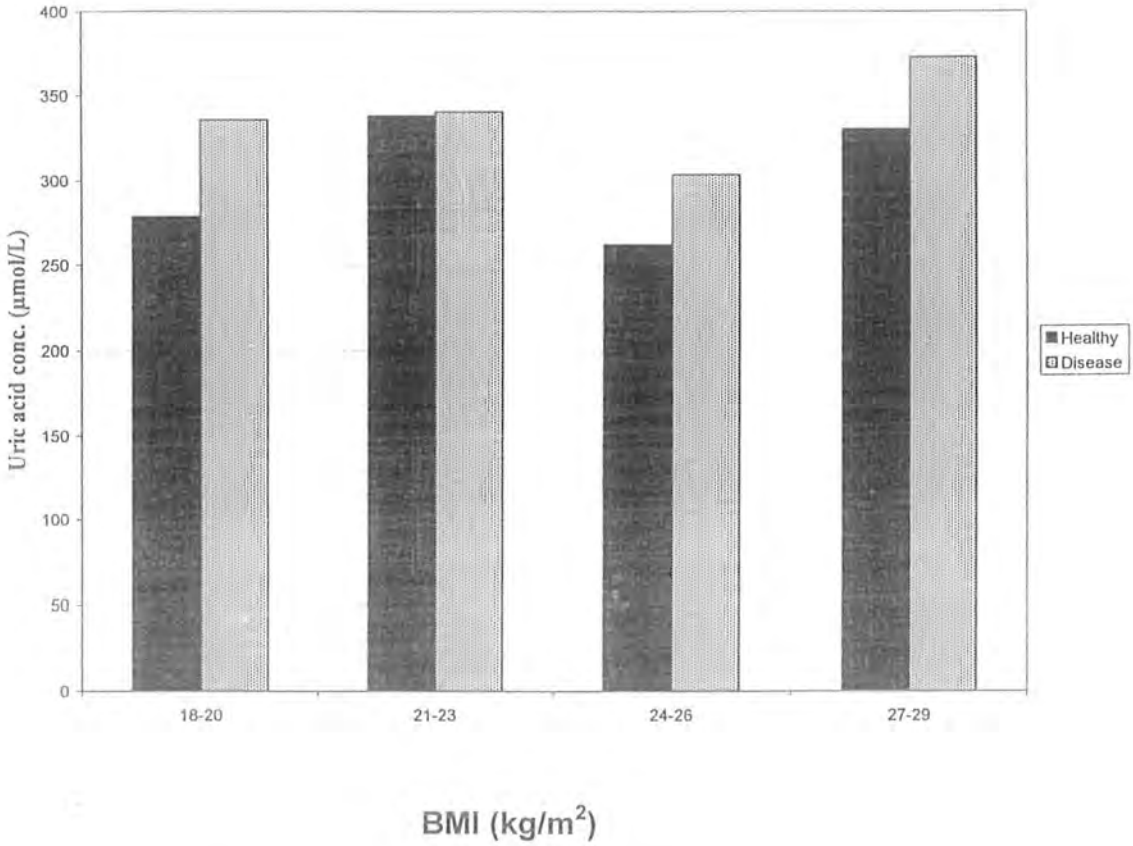

Fig. 3.4 **Effect of different levels of BMI on serum uric acid level in healthy and coronary heart disease patients**

3.2 Estimation of lipid profile

The concentrations of cholesterol, triglyceride, HDL and LDL were estimated in serum samples of both groups. The mean ± SD values of cholesterol, Tg, HDL and LDL were normal in both the groups (Healthy and CHD subjects) and given in Table 3.7.

Table 3.7 Lipid profile of healthy and coronary heart disease patients

Lipid Profile	Normal Values (mg/dl)	Mean ± SD	
		Healthy individuals	CHD patients
Cholesterol	150-200	175.20 ± 19.17	180.25 ± 18.10
Triglyceride (Tg)	upto 200	123.60 ± 25.09	129.13 ± 26.04
HDL Cholesterol	25-50	44.46 ± 4.04	41.20 ± 3.64
LDL Cholesterol	upto 180	105.68 ± 16.39	110.23 ± 15.06

DISCUSSION

CHAPTER # 4

DISCUSSION

The present study was aimed for the determination of serum uric acid level in healthy and coronary heart disease patients and to find out correlation between SUA and CHD. A correlation between SUA and CHD was made by comparing the serum uric acid values of healthy and coronary heart disease patients. Out of 100 male subjects aged between 35-55 years, 50 were normal healthy subjects and 50 were cardiac patients. The coronary heart disease patients were diagnosed by cardiologist with the help of angiography, thallium scan, exercise stress test (ETT) and myocardial infarction. Different characteristics including various risk factor and eating habits were also studied. Among healthy individuals, 16% were fatty, 44 % vegetarian and 40 % were mixed while among coronary heart disease patients, 28% were fatty, 32% vegetarian and 40% were found to be mixed individuals.

The mean ± SD values of age for healthy and disease patients were 42.40 ± 5.38 and 44.26 ± 4.94. There was no significance difference between mean ± SD of age, height, weight and BMI of healthy and CHD patients, thus their effects were considered negligible on CHD events and only SUA was found to be a major risk factor in developing CHD. Thus serum uric acid may be an independent risk factor and have positive correlation with CHD. Other factors like age, weight, height, BM and dietary habits thus not considered as risk for CHD in the present study.

There was found a positive correlation between elevated serum uric acid level and coronary heart diseases. Epidemiological studied suggested that there is an association between SUA and CHD (Klein *et al.*, 1973; Persky *et al.*, 1979; Beard, 1983; Freedman *et al.*, 1995 and Wannamethee *et al.*, 1997). Culleton *et al.* reported in 1999 data from the Framingham study concerning the role of SUA as an independent risk factor in CHD. An epidemiological link between elevated SUA and an increased CHD risk has been recognized for many years (Kohn and Prozan, 1959; Beard, 1983). Observational studies showed that SUA concentrations are higher in patients with established CHD compared with healthy controls (Torun *et al.*, 1998). The association between these risk factors and incidence of CHD has been established by several prospective epidemiological studies in the United State and Europe. These studies demonstrate a consistent association among characteristics observed at one point in time in apparently healthy individual with the subsequent incidence of CHD in those individuals. So as a result of these associations, each characteristic has been termed a risk factor for CHD. High SUA level is a risk factor in CHD (Galvan *et al.*, 1995). The issue of whether SUA is an independent predictor of mortality in patients with CHD or only represents an indirect marker of adverse out come, by reflecting the association between SUA and other CHD risk factor, is a mater of controversy (Freedman *et al.*, 1995; Wannamethee *et al.*, 1997; Fang and Alderman, 2000). The study was therefore conducted to establish a correlation between serum uric acid and CHD.

Mean ± SD values of uric acid concentration for healthy and CHD patients were found to be 298.28 ± 94.58 and 333.88 ± 105.80, μmol/L respectively which showed the increase in SUA level in CHD events. In experimental and *in vitro* systems, SUA appeared to have the ability to induce inflammatory and vascular mechanisms that contribute to rather than protect against the development of CHD (Richard *et al.*, 2003). In CHD patients SUA levels < 303 μmol/L (5.1 mg/dl) compared with those of SUM levels > 433 μmol/L (7.1 mg/dl), the mortality rate was increased from 3.4% to 17.1% (five-fold increase). SUA was found to be an independent predictor of mortality in patients with angiographically proven CHD (Christoph *et al.*, 2002).

Elevated serum uric acid is associated with subjects at CHD risk may count for hyperuricemia that predict the development of CHD in the general population in subjects with hypertension and with pre-existing CHD (Tuttle *et al.* 2002). SUA is not an independent risk factor for CHD after controlling for these other risk factors. Hyperuricemia is therefore considered unless associated with gout or kidney stone (Duffy *et al.* 1981). SUA has a pathogenic role in hypertension and CHD (Richard *et al.* 2003). Most epidemiological evidence suggests a significant, graded, independent and specific association between the level of SUA and CHD morbidity and mortality (Alderman and Michael 2002).

Serum uric acid is linked to the heart disease deaths. Peoples with high levels of uric acid in their blood had an increased risk of dying from heart disease. The presence of

elevated uric acid identifies a sign of greater risk of CHD mortality. That effect is greater in women than in men and considerably greater in African Americans than in whites. Among men, the risk was 77% higher with the highest levels compared with those with the lowest levels. In both men and women, the relationship between SUA and heart-related deaths was only present in people aged 45 and older. People aged 45 to 54 who has high levels of uric acid appeared to have the highest risk. Increased SUA levels are linked to obesity, distorted cholesterol levels and high blood pressure (Mercola 2000).

Increase in serum uric acid in subjects with CHD might therefore reflect a compensatory mechanism to counter the oxidative stress that occurs in these conditions. However this does not readily explain higher SUA level in patient with CHD are generally associated with worse out come (Ames *et al.*, 1981).

Serum uric acid is not an independent risk factor for CHD. There is much controversy concerning the role of SUA as an independent risk factor in the development of coronary heart disease because SUA is related to many of the established etiologic risk factors for CHD. This review finds little support for an independent causal role for serum uric acid in the development of CHD (Wannamethee 2001).

Serum uric acid correlation was made among age, body mass index (BMI) and dietary habits of both the groups. SUA level was determined in four different age groups from 35-55 years in healthy as well as CHD patients. SUA level was found to be 303.12, 349.00, 337.35 and 378.00 μmol/L in four different age groups viz. 35-39, 40-44, 45-49

and 50-54 years of CHD patients which was higher than SUA level of healthy individuals in these groups. SUA level was found to be 268.26, 298.30, 313.90 and 350.00 µmol/L in 35-39, 40-44, 45-49 and 50-54 years age groups of healthy individuals. It was also found that SUA level was increased with an increase in age in both the healthy as well as CHD patients. Lowest SUA level was found to be in 35-39 years of age group (first quartile) in both the healthy as well as CHD patients while highest SUA concentration was found to be in 50-54 years of age group (fourth quartile) of both healthy and CHD patients (Table 3.5). There is a marked increase in the risk of CHD with increasing age. Every eighth man 40-44 years of age have some form of CHD; the percentage of men developing CHD increased with age to approximately every sixth man aged 45-49 years, every fifth man aged 50-54 years, and every fourth man 55 years of the age or older (Castelli and Leaf, 1985).

It is recommended that clinicians first classify patients by body mass index (BMI), calculated as weight in Kg divided by the square of height in meters, with overweight defined as a BMI of 25.0 – 29.9 Kg/m^2 and obesity as a BMI at least 30 Kg/m^2 (NIH, 1998). Obesity is associated with CHD, hypertension, stroke and diabetes mellitus (Bray, 1985; Van, 1979). In men at least height appears to be independent transmissible risk factor for CHD (Kee *et al.*, 1997). SUA level was determined in four different ranges of BMI viz. 18-20, 21-23, 24-26 and 27-29 in healthy as well as CHD patients. It was found that SUA level increased with an increase in BMI in both the healthy as well as CHD patients. Lowest SUA was found to be in third quartile (BMI, 24-26 Kg/m^2) in both the healthy as well as CHD patients (Table 3.6) while highest SUA

was found in overweight persons (fourth quartile) (27-29 Kg/m^2 BMI) having CHD (Fig. 3.4). SUA level was found to be 336.00, 340.66, 303.77 and 372.72 µmol/L in CHD patients of 18-20, 21-23, 24-26 and 27-29 Kg/m^2 of BMI which was higher than the SUA level of healthy individuals in the respective groups (Table 3.6). SUA level was found to be 278.60, 338.27, 261.80 and 330.66 µmol/L in healthy individuals of 18-20, 21-23, 24-26 and 27-29 Kg/m^2 of BMI. A consistent positive association between BMI and SUA was reported in several previous population studies (Goldbourt *et al.*, 1980; Okada *et al.*, 1980; Prior *et al.*, 1966). Our data also indicates a strong association between BMI and SUA. These findings are in line of those of Ying *et al.*, 1997 who reported a strong correlation between SUA and BMI. In addition a significant relationship between weight loss and diet or exercise and decrease in SUA was reported by Nicholls and Scoot, 1972. All these data indicate that BMI has an important influence on SUA level.

Dietary habits of subjects played an important role in the development of CHD and subsequent elevation of SUA. Persons with CHD (28% fatty) showed an increase in SUA level than normal healthy subjects. Fatty patients (overweight) had higher SUA and developed higher CHD chances than normal subjects. The association between SUA and other variables, independent of BMI, pose intriguing questions as to metabolic interrelationships and factors possibility accounting for them, environmental (e.g. dietary) and genetic. Diet played an important role in subsequent CHD formation (Tuomilehto et al., 1988; Okada et al., 1980). Individual who consume a healthier diet might easily be assumed to smokeless, drink less, exercise more and have a more favorable BMI (Miguel *et al.*, 1998).

REFERENCES

CHAPTER # 5

REFERENCES

Alderman and Michael, H. 2002. Uric acid and cardiovascular risk. Curr. Upin. Pharmacol. 2(2): 126-130.

Alderman, M. H. 2001. Serum uric acid as a cardiovascular risk factor for heart disease. Curr. Hypertens. Rep. 3(3): 184-189.

Ames, B. N., Cathcart, R., Schwiers, E. and Hochscein, P. 1981. Uric acid provides an antioxidant defense in human against oxidant and radical causing aging and cancer: a hypothesis. Proc. Natl. Acad. Sci. USA. 78: 6853-6862.

Beard, J. T. 1983. Serum uric acid and coronary heart disease. Am. Heart. J. 106: 397-400.

Bray, G. A. 1985. Complications of obesity. Ann. Intern. Med. 103: 1052-1062.

Brock, D. J. H. 1993. Multifactorial disorder. In: Molecular genetics for the clinician. Cambridge Univ. Press. pp. 223-229.

Brounwald, 1971.Central of myocardial oxygen consumption,physiologic and clinical considering Am.J.Cardiol 27:416

Calbrath, D. F. 1992. Liver and Kidney function, In:Clinical Chemistry. W.B. Saunders Co. pp.246.

Castelli, W. and Leaf, A. 1985. Identification and assessment of cardiac risk-an overview. Cardiol. Clin. 3: 171-178.

Castelli, W. P. 1986. Am. Heart. J. 112: 432-437 (Cited in Skuladottir *et al.*, 1995).

Chadha, S. L. 1998. Urban rural differences in prevalence of coronary heart disease and its risk factor. Current Sciencs. 74(12): 1069-1073.

Chiang, B. W., Perlman, L. V., and Epstein, F. H. 1969. Overweight and hypertension: A review. Circulation. 39: 403.

Christoph B., Hans, J. R., Stefan, B., Gerd, R., Gerd, H., Alexander, D., Klaus-Peter, H. and Jurgen M. 2002. serum uric acid as an independent predictor of mortality in patients with angiographically proven coronary artery disease. Am. J. Cadiol. 89: 12-17.

Cook, D. G., Shaper, A. G., Pocock, S. J. and Kussick, S. J. 1986. Giving up smoking and the risk of heart attack: A report from the British Regional Heart Study. Lanset. 2: 1376-1380.

Cotran, K. and Robins, 1989. The heart. In: Robins pathologic basis of disease. 4th edn. W. B. Saunders international edn. pp. 597-656.

Culleton B. F., Larson, M. G., Kannel, W. B. and Levy, D. 1999. Serum uric acid and risk for CVD and death: the Framingham Heart Study. Ann. Intern. Med. 131: 7-13.

Culleton, B. F. 2001. Uric acid and cardiovascular disease: a renal-cardiac relationship. Curr. Opin. Nephrol. Hypertense. 10(3): 371-375.

Duffy, W. B., Sennekjian, H. O., Knight, T. F., Weinman, E. J. 1981. Management of asymptomatic hyperuricemia. JAMA. 246: 2215-2216.

Fang, J. and Alderman, M. H. 2000. Serum uric acid and cardiovascular mortality: The NHANES I epidemiologic follow-up study1971-1992. JAMA. 283: 2404-2410.

Fayyaz-ud-din, Obaiduallah, S. and Ahmed, I. 1988. Some biochemical incidence of diabetic vascular disease. PJMR. 27: 81-84.

Fieback, N.H., Visculi, C. M. and Horvitz, R. I. 1990. Differences between women and men in survival after myocardial infarction. Biology or Methodology? J.A.M.A. 263: 1092-1096.

Flack, J. M. and Wiist, W. H. 1991. Epidemiology of hypertension and hypertensive target-organ damage in the United States. J. Assoc. Acad. Minor. Phys. 2(4): 143-150.

Franse, L. V., Pahor, M., Di, B. M., Shorr, R. I., Wan, J. Y., Somes, G. W., Applegate W. B. 2000. Serum uric acid, diuretic treatment and risk of cardiovascular events in the systolic hypertension in the Elderly Program. J. Hypertens. 18: 1149-1154.

Freedman D. S., Williamson D. F., Gunter E. W. and Byers, T. 1995. Relation of serum uric acid to mortality and IHD. The NHANES 1. Epidemiologic Follow-up study. Am. J. Epidemiol. 141: 637-644.

Fukiyama, K., Kimura, Y., Wakugami K. and Muratani, H. 2000. Incidence and long-term prognosis of initial stroke and acute myocardial infarction in Okinawa, Japan. Hypertens. Res. 23(2): 127-135.

Galvan, A. Q., Netali, A., Baldi, S., Frascerra, S., Sanna, G., Ciociaro, D., Ferrannini, E. 1995. Effect of insuline on uric acid excretion in human. Am. J. Physiol. 268: E1-E5.

Golberg, R.J., Gore, J. M., Gurvitz, J. H. 1989. The impact of age on incidence and prognosis of initial acute myocardial infarction: The Worcester Heart Attack. Study. Am. Heart. J. 117: 543-549.

Goldberg, R. J. 1992. Coronary heart disease: Epidemiology and risk factor. In: Ockene, I. S. and Ockene, J. K., eds. Prevention of coronary heart disease. Little, Brown and Comopany, Boston/Toronto/London. pp. 3-39.

Goldbourt, U., Medalie J. H., Herman, J. B. and Neufeld, H. N. 1980. Serum uric acid: correlation with biochemical, anthropometric, clinical and behavioral parameters in 10000 Israeli men. J. Chron. Dis. 33: 435-443.

Gordon, T., Kannel W. B., Castilli, W. P. 1982. Multiple risk functions for predicting coronary heart disease concept, accuracy and application. Am. H. J. 103: 1031-1039.

Guyton, 1971. Basic human physiology. Normal function and mechanism of disease. W. B. Saunder Company. pp. 643-645.

Heger, J. W., Roth, R. F., Niemann, J. T., Criley, J. M. 1994. Cardiology. 3rd edn. Williams and Wilkins. Baltimore. pp. 130-307.

Kannel W. B., Neaton J. D., Went W. D., Kennedy R.D. 1986. Overall and CHD mortality rates and major risk factors in 325348 screened for MRF. Am.H.j.112: 825-836.

Kannel, W. B. 1978. Hypertension blood lipid, and cigarette smoking as co risk factors for coronary heart disease. Ann. N. Y. Acad. Sci. 128.

Kannel, W. B. 1981. Update on the rate of cigarette smoking in coronary heart disease. Am. Heart. J. 103: 319-328.

Kannel, W. B. 1987. Metabolic risk factors for coronary heart disease in women: perspective from the Framingham study. Am. Heart. J. 114: 413-419.

Kannel, W. B. and Schatzkin, A. 1983. A risk factor analysis. Prog. Cardiovas. Disease. 16: 3009-3012.

Kannel, W. B., D'Agostino, R. B. and Belanger, A. G. 1987. Fibrinogen, cigarette smoking and the risk of cardiovascular disease. Insight the Framingham study. Am. Heart. J. U3: 1006.

Keating, M. T. and Sanguinetti, M. C. 1996. Molecular genetic insights into cardiovascular disease. Science. 272: 681-685.

Kee, F., Nicaud, V., Tiret, L., Evans, A., O'Reilly, D. and de Backer, G. for the EARS group. 1997. Short stature and heart disease: Nature or nurture? Int. J. Epidemiol. 26: 748-756.

Klein, R. N.1973. Serum uric acid: its relation to coronary artery disease, risk factor and cardiovascular disease: Evans. County. Georjia. Arch. Intern. Med. 132: 401-410.

Kleinman, J. C., Bayers, I., Williamson, D. F., Mardans, J. and Anda, R. F. 1988. Mortality among diabetics in a national sample. Am. J. Epidemiol. 128(2): 389-401.

Kohn P. M. and Prozan, G. B. 1959. Hyperuricemia: relationship to hypercholesterolemia and acute MI. JAMA, 170: 1909-1915.

Kuzuya, M., Ando, F., Iguchi, A and Shimokata, H. 2002. Effect of aging on serum uric acid levels: Longitudinal changes in a large Japanese population group. J. G. Biol. Med. Sci. 57(10): 660-664.

Leal-Pinto, E., Cohen, B. E. and Abramson, R. G.1999. Functional analysis and molecular modeling of a cloned urate transporter/channel. J. Membr. Biol. 169: 13-27.

Lipson, L. G. 1984. Special problem in treatment of hypertension in patients with diabetes mellitus. Arch. Intern. Med. 144: 1829-1831.

Longo-Mbenza, B., Luila, E. L., Mbete, P. and Vita, E. K. 1999. Is hyperuricemia a risk factor of stroke and cornary heart disease among Africans? Int. J. Cardiol. 71(1): 17-22.

Lu, P., Hu, D., Lu, J., Wang, W. and Chen, B. 2002. The association between uric acid and coronary heart disease. Zhonghua-Nei-Ke-Za-Zhi. 41(8): 526-529.

Malik, R., Bukhari, A. H. 1995. A study of blood pressure, serum lipid, cholesterol and Tg in diabetic patient. PJMC. 34: 226-228.

Many, A., Hubel, C. Q. A. and Roberts, J. M. 1996. Hyperuricemia and xanthine oxidase in preeclampsia, revisited. Am. J. Obster. Gynecol. 174: 288-291.

Mercola, S. J. 2000. High Uric Acid Linked To Heart Disease Deaths. American Medical Association, 283: 2404-2410.

Miguel, A. M. G., Jose, F. G., Fernando, S. I., Pablo, L. C., Jose, J. J. M. and Ramon, G. V. 1998. Life style factor associated with changes in serum lipid in a follow-up study of cardiovascular risk factor.

National institutes of health, National Heart, Lung, and Blood Institute 1998. Clinical Guideline on the identification, Evaluation, and Treatment of Overweight and obesity in adults: The Evidence Report. Rockville, Md: 1-228.

Nicholls, A., Scoot, J. T. 1972. Effect of weight-loss on plasma and urinary levels of uric acid . Lancer; ii: 1223-1224.

Okada M, Takeshita M, Ueda K, Omae T and Hirota Y 1980. Factors influencing the serum uric acid level . A study based on population survey in Hisayama town , Kyushu , Japan . J Chron Dis;33:607-612.

Perez-Ruiz F., Clabozo M., Herrero-Beites A. M., Garcia-Frauskin J. and Pijoan J. I. 2000. Improvement of renal function in patients with chronic gout after proper control of hyperuricemia and gouty bouts. Nephrol. 86: 287-291.

Persky, V. W., Dyer, A. R., Idris-Soven, E., Stamler, J., Shekelle, R. B., Schoenberger, J. A., Berkson, D. M. and Lindberg, H. A. 1979 .Uric Acid :a risk factor for CHD .Circulation 59:969-977.

Polonsky, K. S., Given, B. D. and Hirsch, L. J. 1988. Abnormal patterns of insuline secretion in NIDDM. N. Engl. J. Med. 318: 1231-1239.

Prior, I. A. M., Rose, B. S. and Harvey, H. P. B. 1966. Hyperuricaemia, gout and diabetic abnormality in Polynesian people. Lancet. 1: 333-338.

Qadri, M. R., Ali, A., Hamid, S., Sabir, A. W. and Chaudri, T. A. 1996. Effect of dietary intake of different fats on morbid obesity in male. Hmd. Med. 39 (2): 82-91.

Reaven, G. M. 1988. Role of insuline resistance in human disease. Diabetes. 37: 1595-1607.

Richard, J. Johnson, Duk-Hee, K., Daniel, F., Salah, K., Johan K., Susumu W., Katherine R. T., Bernardo Rodriguez-Iturbe, Jaime Herrera-Acosta, Marilda, M. 2003. Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal diseases? Hypertension. 41: 1183-1190.

Robinson, K., Conroy, R. M. and Mulcahy, R. 1989. When does the risk of acute coronary heart disease in ex-smokers fall to that in non-smokers? A retrospective study of patients admitted to hospital with a first episode of myocardial infarction or unstable angina. Br. Heart. J. 62: 16-19.

Robinson, K., Conroy, R. M., Mulcahy, R. and Hickey, N. 1988. Risk factors and in hospital course of first episode of myocardial infarction or acute coronary insufficiency in women. J.A.C.C. 11: 932-936.

Saud, M. F., Knoller, W. C. and Benett, D. 1991. A two step model for development of NIDDM, AJM. 90: 229-334.

Silberg, J. S., Wlodarczyk, J., Fryer, J., Ray, C. D. and Hensley, M. J. 1998. Correction for biases in a population based study of family history and coronary heart disease. The Newcastle Family History Study. I. Am. J. Epidemiol. 147: 1123-1132.

Simpson, N. E., Multifactoria, L. Inheritance. 1984. A possible hypothesis for diabetes. Diabetes 1; pp 462-471.

Snowden, C. B., McNamara, P. M., Garrison, R. J. 1982. Predicting coronary heart disease in siblings: A multivariate assessment. Am. J. Epidemiol. 115: 217-222.

Tabayashi, K., Suzuk, Y., Nagamine, S., Ito, Y., Sekino, Y., Mohri, H. 1991. A clinical trial of allopurinol (zyloric) for myocardial protection. J. Thorac. Cardiovasc. Surg. 101: 713-718.

Torun, M., Yardim, S., Simsek, B. and Burgaz, S.1998. Serum uric acid levels in CVD. J Clin Pharm Ther. 23: 25-9.

Tuomilehto, J., Zimmet, P., Wolf, E., Taylor, R., Ram, P. and King, H. 1988. Plasma uric acid level and its association with diabetes mellitus and some biological parameters in a biracial population of Fiji .Am J Epidemiol; 127:321-336.

Tuttle, K. R., Johnson, R. J. Short, R. A. 2002. Microalbuminuria and serum uric acid levels as predictors of cardiovascular events over five year. J. Am. Soc. Nephrol. 13: 442A. Abstract.

Van, I. T. B. 1979. Obesity: Adverse effects on health and longevity. Am. J. Clin. Nutr. 23: 2723-2733.

Vaziri, N. D., Freel R. K., Hatch M. 1995. Effect of chronic experimental renal in sufficiency on urate metabolism. J. Am. Soc. Nephrol. 6: 1313-1317.

Wannamethee, S. G. 2001. Serum uric acid is not an independent risk factor for coronary heart disease. Curr. Hypertens. Rep. 3(3): 1990-1996.

Wannamethee, S. G., Shaper A. G., Whincup, P. H. 1997. Serum urate and the risk of major coronary heart disease events .Heart. 78: 147-153.

Waring, W. S., Webb, D. J., Maxwell, S.R. J. 2000. Effect of local hyperuricemia on endothelial dysfunction in the human forearm vascular bed. Br. J. Clin. Pharmacol. 49: 511.

Weber, M. A., Neutal, M. B. and Cheung, D. G. 1989. Hypertension in the age: A pathophysiologic basis for treatment. Am. J. Cardiol. 63: 25-32.

Winniford, M. D., Whellan, K.R. and Kremers, M. S. 1986. Smoking induced coronary vasoconstriction in patients with atherosclerosis coronary artery disease. Evidence for adrenergically mediated alterations in coronary artery tone. Circulation. 73: 612.

Wu, X, Muzny, D. M., Lee, C. C. and Caskey, C. T. 1992. Two independent mutational events in the loss of urate oxidase during hominoid evolution. J. Mol. Eyol. 34: 78-84.

ANNEXURE

Body Mass Index Chart

Directions: Find your weight in pounds or kilograms at along the top. Find your height along the left. Your BMI is where they intersect. This chart does not apply athletes, children and pregnant or lactating women.

WEIGHT lbs	100	105	110	115	120	125	130	135	140	145	150	155	160	165	170	175	180	185	190	195	200	205	210	215
kgs	45.5	47.7	50.0	52.3	54.5	56.8	59.1	61.4	63.6	65.9	68.2	70.5	72.7	75.0	77.3	79.5	81.8	84.1	86.4	88.6	90.9	93.2	95.5	97.7
HEIGHT in/cm																								
5'0" - 152.4	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
5'1" - 154.9	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
5'2" - 157.4	18	19	20	21	22	22	23	24	25	26	27	28	29	30	31	32	33	33	34	35	36	37	38	39
5'3" - 160.0	17	18	19	20	21	22	23	24	24	25	26	27	28	29	30	31	32	32	33	34	35	36	37	38
5'4" - 162.5	17	18	18	19	20	21	22	23	24	24	25	26	27	28	29	30	31	31	32	33	34	35	36	37
5'5" - 165.1	16	17	18	19	20	20	21	22	23	24	25	25	26	27	28	29	30	30	31	32	33	34	35	36
5'6" - 167.6	16	17	17	18	19	20	21	21	22	23	24	25	25	26	27	28	29	29	30	31	32	33	34	34
5'7" - 170.1	15	16	17	18	18	19	20	21	22	22	23	24	25	25	26	27	28	29	29	30	31	32	33	33
5'8" - 172.7	15	16	16	17	18	19	19	20	21	22	22	23	24	25	25	26	27	28	28	29	30	31	32	32
5'9" - 175.2	14	15	16	17	17	18	19	20	20	21	22	22	23	24	25	25	26	27	28	28	29	30	31	31
5'10" - 177.8	14	15	15	16	17	18	18	19	20	20	21	22	23	23	24	25	25	26	27	28	28	29	30	30
5'11" - 180.3	14	14	15	16	16	17	18	18	19	20	21	21	22	23	23	24	25	25	26	27	28	28	29	30
6'0" - 182.8	13	14	14	15	16	17	17	18	19	19	20	21	21	22	23	23	24	25	25	26	27	27	28	29
6'1" - 185.4	13	13	14	15	15	16	17	17	18	19	19	20	21	21	22	23	23	24	25	25	26	27	27	28
6'2" - 187.9	12	13	14	14	15	16	16	17	18	18	19	19	20	21	21	22	23	23	24	25	25	26	27	27
6'3" - 190.5	12	13	13	14	15	15	16	16	17	18	18	19	20	20	21	21	22	23	23	24	25	25	26	26
6'4" - 193.0	12	12	13	14	14	15	15	16	17	17	18	18	19	20	20	21	22	22	23	23	24	25	25	26

- Underweight (BMI less than 18.5)
- Healthy weight (BMI 18.5 to 24.9)
- Overweight (BMI 25 to 29.9)
- Obese (BMI 30 to 39.9)
- Extremely obese (BMI 40 and above)