DISSERTATION ON

STUDY OF SERUM GAMMA GLUTAMYL TRANSFERASE LEVELS IN FEMALE PATIENTS WITH METABOLIC SYNDROME

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FOR THE EXAMINATION IN MARCH 2009

CERTIFICATE

Certified that this dissertation on ***" STUDY OF SERUM GAMMA GLUTAMYL TRANFERASE LEVELS IN FEMALE PATIENTS WITH METABOLIC SYNDROME"*** *is the Bonafide work done under my guidance by* ***DR.K.P.MEKHALA****, appearing for the* ***Branch XIII M.D. BIOCHEMISTRY*** *Examination of the* ***TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY*** *in* ***MARCH 2009****.*

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STUDY OF SERUM GAMMA - GLUTAMYL TRANSFERASE LEVELS IN FEMALE PATIENTS WITH METABOLIC SYNDROME

INTRODUCTION :

Metabolic syndrome is a combination of Medical disorders that increase the risk of developing Cardiovascular disease and Diabetes[1]. It affects a great number of people and prevalence increases with age. Metabolic syndrome is also known as syndrome-X, Insulin Resistance syndrome, Raeven's syndrome, CHAOS (Australia) or Metabo (Japan).

GGT is a Potential biomarker for preclinical development of artherosclerosis because GGT was detected in atheromatous plaques of carotid and coronary arteries triggering oxidation of LDL.

Signs and Symptoms[2]:

1. Fasting Hyper glycemia – Diabetes Mellitus Type II or Impaired Fasting Glucose, Impaired Glucose tolerance or Insulin resistance.
2. High blood pressure
3. Central obesity (Visceral, Male pattern or Apple shaped Adiposity) Over weight with fat deposits mainly around the waist.
4. Decreased HDL – Cholesterol
5. Elevated Triglycerides

ASSOCIATED FINDINGS :

1. Elevated uric acid levels
2. Fatty Liver (Especially in concurrent obesity) Progressing to non-alcoholic liver diseases
3. Polycystic ovarian syndrome
4. Haemochromatosis (Iron over load)
5. Acanthosis Nigricans (skin condition featuring dark patches)

DIAGNOSTIC CRITERIA :

I. **The WHO Criteria (1999) :** Presence of Diabetes Mellitus / Impaired Glucose Tolerance, Impaired fasting glucose and any 2 of the following criteria.

1. Blood pressure > or = 140/90mm of Hg.
2. Central obesity or Body Mass Index > 30Kg /m2
3. Dyslipidemia (Triglycerides > or = 1.695 mmol / lit and
   HDL – C <or = 0.9 mmol / lit for males
   <or =1mmol / lit for females
4. Micro albumin urea – urinary albumin excretion ratio >or =20mg/mt or Albumin creatinine ratio > 30mg /kg/gm

II. NCEP[3] (National Cholesterol Education programme) Adult Treatment Panel III. (2001): Any three of the following criteria

1. Central obesity : waist circumferance

>or = 102cms or 40 inches (Males)

>or = 88cms or 36 inches (Females)

2. Dyslipidaemia : TG >or= 1.695 mmol / l (150mg/dl).

3. Dyslipidaemia: HDL-C <40 mg/dl (male)

< 50mg/dl (females)

4. Blood Pressure : >or =130/85 mm of Hg

5. Fasting plasma glucose >or = 6.1 mmol/l (110mg/dl)

III. UPDATED NCEP[2]

1. Elevated waist Circumference

Men >or = 40 inches (102 cms)

Women >or = 35 inches (88 cms)

2. Elevated Triglycerides >or = 150 mg/dl

3. Decreased HDL – C < 40 mg / dl in Males

< 50mg/dl in females

4. Elevated Blood pressure >or = 130/85 mm of Hg or use of medication for Hypertension.

5. Elevated fasting Glucose of 100 mg/dl (5.6 mmol /litre) or use of medication for Diabetes – Mellitus

ETIOLOGY

The Cause of metabolic syndrome is unknown

The Pathophysiology is extremely complex. Most patients are older, obese, sedentary and have a degree of insulin resistance. The most important factors in order are

(i) Aging

(ii) Genetics

(iii) Life-style, i.e, Low physical activity and excess caloric Intake.

(iv) Some have pointed to oxidative stress due to variety of causes including 'INCREASING URIC ACID LEVELS'[4] caused by dietary fructose.

However number of markers of systemic Inflammation including C - Reactive protein, Fibrinogen, Th-b, Tumor Necrosis factor Alpha (TNF α) are also increased.

Who is at risk for Metabolic syndrome[5]?

(i) A large waist line or abdominal obesity.

(ii) Lack of physical activity

(iii) Insulin resistance.

Other groups who are at increased risk of developing metabolic syndrome include.

1. People with a sibling or parent with diabetes
2. People with personal history of diabetes
3. People with a personal history of polycystic ovarian syndrome.

RISK for Heart diseases : Having metabolic syndrome increases risk for heart diseases. Heart disease risk can be divided into short term risk (risk for having a heart attack or dying of heart disease in the next 10 yrs) and Long term risk (risk for developing heart disease over Lifetime).

According to the June issue of "Human Molecular Genetics" (By Kevin Mckeever June 19 2008) at Washington University School of Medicine found the Variation on the CD-36 Gene located in part of Chromosome – 7, previously associated with metabolic syndrome[6].

EPIDEMIOLOGY [7]:

About 47 million adults in United States (25%) have metabolic syndrome. Metabolic syndrome is common in African American Women than in African American men. It is known in Mexican American women than in Mexican American men. Mexican American have the highest rate of metabolic syndrome.

(31.09%) followed by Caucasians 23.8 percent and African American 21.6 percent. South Asians have an increased risk for Metabolic syndrome.

According to American Heart Association 2008 update the age adjusted prevalence of metabolic syndrome for adults is 23.7 percent. The prevalence is 42 percent for age greater than 70 yrs and 43.5 percent for age group 60-69 yrs and 6.7 percent among 20-29 yrs. People reporting other race of Ethnicity prevalence is 20.3 percent[8].

GGT

Gamma Glutamyl Transferase is a cell surface protein contributing to extra cellular catabolism of glutathione (GSH)[9] The enzyme is produced in many tissues, but most GGT in serum in derived from liver. In the serum GGT is primarily carried with lipoprotein and albumin.

One hypothesis for the relation of GGT levels and Vascular disease hold that GGT itself is PROATHERO GENIC[10]. GGT has been reported to occur in atherosclerotic plaques[11], which might support this hypothesis. The origin of GGT in plaques could be through influx of lipoproteins. One of the products of GSH hydrolysis produced by GGT is cystenyl – Glycine which can generate super oxide

anion radicals through its interaction with free ion. This would promote atherogenesis via LDL oxidation

GGT is present in serum and all cells except muscles[21] . In the cell it is located in cell membrane and less in cytosol. Its functions are that it transports amino acids and peptides across cell membrane into cells. It is involved in glutathione metabolism.

clinical significance of GGT :

Origin of GGT is primarily from the Hepatobiliary system. So it's level is increased in all forms of liver disease.

a. Earliest and highest elevation in obstructive jaundice, cholangitis and cholecystitis. Increase is 5-30 times the normal levels.
b. Infectious hepatitis Increase is 2-5 times normal levels.
c. Early and high elevations in individuals with primary or secondary neoplasms.
d. Increase of 2-5 times normal GGT activity in fatty liver.
e. Transient increase in drug intoxications is observed.
f. Acute and chronic pancreatitis and in carcinoma head of pancreas obstructing biliary flow – Increase is 5-15 times of normal activity.

g. Increase in levels of serum GGT in alcoholic cirrhosis and heavy drinkers.

h. In myocardial infarction – GGT is normal. But increase may occur by 4^{th} hour peak in 4 days. Why? Secondary to liver damage due to cardiac insufficiency.

i. Increase in serum levels of GGT – elevated in drug intake (Phenytoin, Phenobarbitone) due to induction of new enzymes activity by anticonvulsants.

j. High levels of GGT are present in prostate (50% higher activity in sex of men than women.

k. At times increased in prostatic malignancy.

l. Irradiation of tumors. Increase in GGT activity in serum.

m. Origin of GGT in urine is from kidneys and gut.

Since GGT is Proatherogenic, it is essential to find the levels of Serum Gamma Glutamyl Transferase in individuals with Metabolic Syndrome which is already associated with (high) cardiac risk factors.

OBJECTIVE

To study the level of GGT in serum of female patients with Metabolic Syndrome.

MATERIALS AND METHODS

The dissertation study was carried out in the Hypertensive Out-Patient Department of Thanjavur Medical College Hospital.

My study group comprises of 100 females with Metabolic syndrome according to 3/5 criteria of National Cholesterol education programme (NCEP), in whom fasting serum levels of Gamma-Glutamyl Transferase levels were estimated.

The patients were identified as having Metabolic syndrome according to the following criteria.

1. Elevated waist circumference of >or= 35 inches (88 cms)
2. Elevated Triglycerides >or=150 mg/dl
3. Decreased HDL – C<or=50 mg/dl
4. Elevated Blood pressure of >or= 130/85 mm of Hg.
5. Elevated fasting Glucose of >or= 100mg/dl.

All the people in the study group were enquired by the following

Questionnaire

1. Name Address
2. Age
3. Gender
4. Diet
5. Occupation
6. Religion
7. Complaints
8. Past History suggestive of

   DM/BA/TB

   PIH or GDM
9. Personal History :

   H/O Alchohol in take

   Menstrual History
10. Family History of Obesity,

    HT, DM, BA, TB, Stroke,

    CV diseases.

Treatment History : What Anti Hypertensive drug is she on? and how long?

General Examination

a. Anaemia

b. Jaundice

c. Xanthalesma

d. Cyanosis

e. Clubbing

f. Pedaloedema

g.Lymphadenopathy

h. Pulse rate

i. Blood pressure

Height :

Weight :

Body mass index

Waist circumference

Measurement

Systemic Examinations.

1. Cardiovascular System
2. Respiratory system
3. Abdomen
4. Central Nervous system

METHODOLOGY

Study Group Humans

Criteria :- Female Patients in the age group 30 to 75 yrs.

Inclusion Criteria

1. Obesity
2. Hypertension
3. Dyslipidemias over 150 mg/dl TAG Blood
4. Fasting blood sugar More than 106 mg/dl
5. HDL less than 50mg/dl

Exclusion Criteria

1. Liver diseases
2. Renal Diseases
3. Alcoholism
4. Drug in take (Anticoagulants)
5. Males (Prostatic GGT)

METHODOLOGY:

The following parameters were measured by using different methodologies.

Parameters included under study supporting diagnosis of Metabolic syndrome.

I. Gamma – Glutamyl Transferase

II. 1. Fasting Blood Glucose

2. Serum Cholesterol

3. HDL – Cholesterol

4. Serum Triglycerides

5. LDL

6. SG OT

7. SG PT

8. Alkaline Phosphatase

9. Serum Uric acid

10. Serum Urea

11. Serum Creatinine

Estimation of γ – Glutamyl Transferase levels in serum.

Kinetic (SZASZ Method) [49]:

Principle :

γ – Glutamyl transpeptidase catalyses the transfer of Gamma-Glutamyl group from the substrate Gamma-Glutamyl para-Nitroanilide to Glycyll Glycine releasing free P-Nitro aniline which absorbs light at 405nm.

Enzyme activity is proportional to increase in absorbance at 405nm.

GPNA + Glycyl Glycine $\xrightarrow{\gamma - GT}$ L- γ –Glutanyl Glycyl Glycine + P-Nitro aniline

GPNA = L- γ – Glutamyl – P-Nitroanilide.

Reagent contents:

Reagent 1 (substrate)

Glycyl Glycine	94 mmol/L
L- γ -Glutamyl – P-Mitroanilide	3.2 mmol/L

Reagent 1A (Buffer)

Tris Buffer (PH-8.20)	200mmol/L
Surfactant	0.2%

Reagent Preparation :

3ml of reagent 1A was added to one bottle of reagent 1 and mixed gently by swirling till it dissolved completely.

Sample Material : SERUM

Procedure :

General System parameters.

The Instrument was set with the following parameters

Reaction type	:	Kinetic
Reaction slope	:	Increasing
Wavelength	:	405 nm
Flow cell temp.	:	30^{o}C
Delay time	:	60 sec
No.of Readings	:	4
Internal	:	60 Secs
Sample vol.	:	100 µl
Reagent vol.	:	1 ml

Path length : 1 cm

Factor : 11.11

Zero setting with distilled water.

A Test tube was taken. 1ml of reconstituted reagent was dispersed into it. Then 100µl of the test serum was added, mixed and read immediately.

Linearity :

This method is linear upto 189u/l

Reference values :

Serum γ – Glutamyl Transferase :

Males - 7-34 u/l (30^oC)

Females - 4-25 u/l (30^oC)

ESTIMATION OF GLUCOSE IN FASTING SERUM :

Method : Glucose Oxidase – Peroxidase Method[49]

Principle :

Glucose is oxidised to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenyl and 4-amino antipyrine by the catalystic action of peroxidase to form a red colored quinonein dye complex.

Intensity of colour formed is directly proportional to amount of glucose present in the sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconate} + H_2O_2$$

$$H_2O_2 + 4 \text{ Amino antipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Red Quinoneine dye} + H_2O$$

Reagent Contents :

L1 : Glucose reagent : 4 x 250 ml

L2 : Buffer reagent : 10ml

Glucose standard (100mg/dl) : 5 ml

Reagent Preparation :

2.5ml of Buffer reagent (L2) was added to 250ml of distilled water. The contents of one bottle of Glucose reagent (L1) was emptied into it and mixed by gently swirling and allowed to stand at room temperature for 30 minutes. The working reagent is stable for 60 days when stored at 2-8^{o}C.

Sample Material : *Fasting Serum*

Procedure :

The instrument was set with the following parameters

Reaction type	:	End point
Reaction slope	:	Increasing
Wavelength	:	505 nm
Incubation Temp.	:	37^{o}C/R.T
Sample Vol.	:	10 minutes / 30 minutes
Reagent Vol.	:	1.0 ml
Standard concentration	:	100 mg / dl
Zero setting with	:	Reagent Blank
Linearity	:	500 mg /dl

The reagent, distilled water standard and sample were pipetted into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T) as following:-

Addition sequence	**B(ml)**	**S (ml)**	**T (ml)**
Working Reagent	1.0	1.0	1.0
Distilled water	0.01	--	--
Glucose standard	--	0.01	--
Sample	--	--	0.01

All test tube contents were mixed well, incubated at 37^{o}C for 10 minutes. The absorbance of standard (Abs.S), Test sample (Abs.T) were measured against blank within 60 minutes. At 505 nm (green filter).

Calculation :

$$\text{Total Glucose in mg/dl} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 100$$

Linearity :

This procedure is linear upto 500mg/dl.

Reference value :

Serum : Glucose Fasting level = 74-106mg/dl.

ESTIMATION OF SERUM TRIGLYCERIDES.

Method : Enzymatic calorimetric method[49].

Principle :

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

Glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol – 3 – Phosphate+ ADP

Glycerol – 3 – Phosphate + O2 + GPO $\longrightarrow$ Dihydroxy acetene

Phosphate + H_2O_2

2 H_2O_2 + 4 – amino antiprine + ADPS $\xrightarrow{\text{Peroxidase}}$

Red Quinone + 4 H_2O

GPO = Glycerol – 3 – Phosphate Oxidase

ADPS = N-Ethyle – N – Sulfopropyl – n – aniside .

The intensity of purple coloured complex formed during the reaction is directly proportional to the trigylceride concentrate in the sample and is measured at 546 nm.

Reagents :

Reagent 1 (Enzymes / Chromogen)

Lipoprotein Lipase	> or = 1100 u/L
Glycerol Kinase	> or = 800 u/L

Glycerol – 3 – Phosphate Oxidase	> or = 5000 u/L
Peroxides	> or = 300 u/L
4 – Amino antipyrine	> or = 0.7 mmol / L
ATP	> or = 0.3 mmol/L

Reagent 1 A (Buffer)

Pipes Buffer, PH 7.50	50 mmol/L
ADPS	1 mmol/L
Magnesium Salt	15 mmol/L
Standard (Triglycerides 200 mg/dL)	
Glycerol (Trig. Equivalent)	2g/L

Reagent Reconstitution :

The reagents are allowed to attain room temperature. The contents of one bottle of reagent 1 were dissolved with one bottle of reagent 1 A, and mixed by gentle squirling and used for 5 minutes.

Reconstituted Reagent storage and stability. The reconstituted reagent is stable for 6 weeks what stored at $2^{o}C$ - $8^{o}C$.

Procedure :-

The samples and the reconstituted reagent were brought to room temperature prior to use. The instrument was set with the following parameters.

General system parameters :

Reaction Type : End point

Reaction Slope : Increasing

Wave length : 546 (520-570nm)

Flow cell Temperature : $30^{o}C$

Incubation : 5 min at $37^{o}C$

Sample Volume : 10 µl

Reagent Volume : 1ml

Std. Concentration : 200 mg / dl

Zero setting with : Reagent Blank

The reconstituted reagent, standard and sample were dispensed into test tubes as follows :

	Blank (ml)	**Standard (ml)**	**Test (ml)**
Reconstituted Reagent	1 ml	1 ml	1 ml
Standard	---	10 µl	
Sample			10 µl

The test tubes are incubated at 37°C for 5 minutes. Mixed well and read at 546nm. The final colour was stable for 30 minutes.

Linearity :

The method is linear upto 1000 mg/dl.

Reference value for Triglycerides

Serum / Plasma

Females	:	20 - 29 yrs	-	37 – 144mg/dl
		30 – 39 yrs	-	39 – 176mg/dl

ESTIMATION OF SERUM CHOLESTEROL

Method : Enzymatic Method[49]

Principle :

$$\text{Cholesterol Ester} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholesterone} + H_2O_2$$

$$2H_2O + \text{Phenol} + 4\text{–Amino Antipyrine} \xrightarrow{\text{Peroxidase}} \text{Red Quinone} + 4 - H_2O$$

The Concentration of Cholesterol in the sample is directly proportional to intensity of Red complex (Red Quinone) which is measured at 500 nm.

Reagents :

Reagent 1 (Enzymes / Chromogen)	
Cholesterol Esterase	≥ 200 u/L
Cholesterol Oxidase	≥ 250 U/L
Peroxidase	≥1000 U/L
4- Amino Antipyrine	0.5 mmol/L

Reagent 1 A (Buffer)

Pipes Buffer PH 6.90	50 mmol/L
Phenol	24 mmol/L
Sodium Cholate	0.5 mmol/L

Standard (Cholesterol 200 mg/dl) :

Cholesterol	2g/L

Storage and stability of the Reagents :

When stored at $2^{o}C$ – $8^{o}C$ and protected from light, the reagents are stable until expiry date on the labels.

Reagent Reconstitution :

The reagents are allowed to attain room temperature. The contents of one Bottle of reagents 1 were dissolved with one bottle of reagent 1A and mixed by gentle swirling.

Reconstituted Reagent storage & Stability :

The reconstituted reagent is stable for 3 months when stored at $2^{o}C – 8^{o}C$.

Procedure :

The sample and reconstituted reagent were brought to room temperature.

The instrument was set with the following parameters.

Reaction Type	:	End point
Reaction Slope	:	Increasing
Wave length	:	500nm (492-550nm)
Flow cell Temperature:		$30^{o}C$
Incubation	:	5 min at $37^{o}C$
Sample Volume	:	10 µl
Reagent Volume	:	1.0ml

Std. Concentration : 200 mg / dl

Zero setting with : Reagent Blank

The reconstituted reagent, standard and sample were dispensed into test tubes as follows;

	Blank (ml)	**Stan (ml)**	**Test (ml)**
Reconstituted Reagent	1 ml	1 ml	1 ml
Standard	---	10 µl	---
Sample	---	---	10 µl

The test tubes are incubated at 37^{o}C for 5 minutes. Mixed well and read at 500nm.

Linearity :

The method is linear upto 500 mg/dl.

<u>Reference value (Serum Cholesterol)</u>

Serum / Plasma Cholesterol

Females	20 - 24 yrs	-	122-216 mg/dl
	25 - 29 yrs	-	128 – 222 mg/dl
	30 – 34 yrs	-	130 – 230 mg/dl

Estimation of LDL CHOLESTEROL BY FRIEDWALD EQUATION [49]

$$(\text{LDL CHOLESTEROL}) = \left[(\text{Total Cholesterol} - \text{HDL Cholesterol})\right] - \left[\frac{\text{Triglycerides}}{5}\right] \text{ mg/dl}$$

Reference Value :

Female : >130 mg/dl

ESTIMATION OF HDL – CHOLESTEROL

Method : **Phosphotungstate method[49].**

Principle :

Chylomicrons, VLDL (Very low Density Liproprotein) and LDL fraction in serum are separated from HDL by precipitating with Phosphotungstic acid and Magnesium chloride. After Centrifugation, the Cholesterol in the HDL fraction, which remains in the Supernatant, is assayed with enzymatic Cholesterol method, using Cholesterol esterase, Cholesterol Oxidase Superoxidase and the Chromogen 4-Aminoantipyrin.

Reagents :

Reagent 1 (Enzymes / Chromogen)

Cholesterol Esterase	> or = 200 u / L
Cholesterol Oxidase	> or = 250 U/L
Peroxidase	> or = 10000 U/L
4 – Aminoantipyrin	.5 mmol / L

Reagent 1 A (Buffer)

Pipes Buffer, PH 6.9	50 mmol / L
Phenol	24 mmol /L
Sodium Cholate	0.5 mmol /L

Reagent 2 (Precipitating Reagent)

Phosphotungstic acid	2.4mmol /L
Magnesium chloride	39 mmol /L

Standard (HDL Cholesterol 50mg/Dl)

Cholesterol 0.5g/L

Reagent Reconstitution :

The reagents are allowed to attain the room temperature. The contents of one bottle of reagent 1 is dissolved into one bottle of reagent 1A, and mixed by gentle swirling till completely dissolved and used after 5 minutes.

Reconstituted Reagent storage and stability :

The reconstituted reagent was stable for 3 months when stored at $2^{o}C$ - $8^{o}C$.

Procedure :

The samples, precipitating reagent 2 and the reconstituted reagent were brought to room temperature prior to use.

The instrument was set with the following parameters.

General System Parameter:

Reaction Type : End point

Reaction Slope	:	Increasing
Wave length	:	500 nm (492 – 550nm)
Flow cell temperature	:	30^{o}C
Incubation	:	5 minute at 37^{o}C
Sample Volume (Supernatant)	:	20 µl
Reagent Volume	:	1.0 ml
Std. Concentration	:	100mg /dl (The std of 50mg /dl is to feel as 100 mg/dl to account for dilution of sample in the precipitation step)
Zero setting with	:	Reagent Blank

	Blank (ml)	**Standard (ml)**	**Test (ml)**
Reconstituted Reagent	1 ml	1 ml	1 ml
Standard	---	10 µl	---
Sample	---	---	10µl

The test tubes were incubated at 37^{o}C for 5 minutes, mixed well and read at 500 nm.

Linearity :

The method is linear upto 500 mg/dl.

Reference Value (Serum Cholesterol)

Serum / Plasma Cholesterol

Female	20-24 yrs	- 122 –216 mg/dl
	25-29 yrs	- 128 – 222 mg/dl
	30-34 yrs	- 130-230 mg/dl.

ESTIMATION OF URIC ACID :

Method : **Enzymatic Method .(Caraway)[49]**

Principles :

Uric acid is converted by uricase into allantoin and hydrogen peroxide which in presence of peroxidase (POD/Oxidises the chromogen to a Red coloured compound which is read at 500 nm (492-550nm). The final colour of the reaction is stable for 15 minutes.

$$\text{Uric acid} + 2\,H_2O_2 + O_2 \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2\,H_2O_2 + \text{Aminoanipyrine} + \text{DHBS} \xrightarrow{\text{PO D}} \text{Red quinone} + H_2O + \text{Hcl}$$

DHBS= 3.5 – Dichloro – 2 Hydroxybenzene Sulfonic acid

POD = Peroxidase.

Reagents :

Reagent 1 (Enzymes / Chromogen)

Uricase	> or = 60 U/L
Peroxidase	> or = 660 u/l
4-Amino antipyrin	0.23 mmol/L

Reagent 1A (Buffer)

Phosphate Buffer, PH 7.5	50 mmol /L
DAB 5	2 mmol /L

Standard (Uric acid 6mg/dl)

Uric acid : 0.06g / L

Storage and stability of the reagents :

When stored at $2^{o}C$ – $8^{o}C$ and protected from light, the reagent are stable until the expiry dates stated on the labels.

Reagent Reconstitution :

The reconstituted reagent was stable for 4 weeks when stored at $2^{o}C$ – $8^{o}C$.

PROCEDURE :

The sample and the reconstituted reagent were brought to room temperature. The instrument was set with the following parameters.

General system Parameters :

Reaction Type	:	End point
Reaction Slope	:	Increasing
Wavelength	:	510 nm (492 – 550nm)
Flow cell temp.	:	30^{o}C
Incubation	:	5 minutes at 37^{o}C
Sample Vol.	:	25 µl
Reagent Vol.	:	1 ml
Std. Concentration	:	6 mg/dl
Zero setting with	:	Reagent Blank.

The reconstituted reagent, standard and sample were dispensed into test tubes as follows.

	Blank (ml)	**Standard (ml)**	**Test (ml)**
Reconstituted Reagent	1 ml	1 ml	1 ml
Standard	---	25 µl	---
Sample	---	----	25 µl

The test tubes were incubated at 37^{o}C for 5 minutes. Mix well and read at 510nm.

The final colour in stable for at least 15 minutes.

Linearity :

The Method is linear upto 25 mg/dl.

Reference Value in serum / Plasma :

Males	:	3.5 – 7.2mg/dl
Females	:	2.6 – 6.0 mg/dl

ESTIMATION OF SERUM AST

Method : Kinetic Method[49]

Principle :

L – Asparate + α – Keto Glutarate $\xrightarrow{\text{GOT}}$ Oxaloacetate + L Glutamate

Oxaloacetate + NADH + H + MDH $\rightarrow$ L – Malate + NADH

AST = Aspartate transaminase + NAD +

MDH = Malate Dehydrogenase

There is a decrease in absorption at 340 nm as NADH is converted to NAD. The rate of decrease in absorbance is measured and is proportional to AST activity in the sample.

Reagents :

Reagent 1 (Enzymes)

1. MDH - > or = 600 u/L
2. LDH - > or = 900 u/L
3. NADH - 0.20 mmol / L
4. α – Ketoglutarate - 12 mmol /L

Reagent 1A (Buffer) :

Tris Buffer : Ph 7.80 88mmol /L

L – Asparatate 260 mmol /L

Storage and stability of Reagents :

When stored at 2°C – 8°C and protected from light stable until expiry date on the label.

Reconstitution of Reagents :

The contents of one bottle of Reagent 1 are dissolved with one bottle of Reagent 1A. Mixed by gentle swirling. The reconstituted reagent is stable for 4 weeks when stored at 2°C – 8°C.

PROCEDURE :

The samples and the reconstituted reagent must be brought to room temperature prior to use.

The instrument must be set with the following parameters.

General System Parameters :

Reaction Type	:	Kinetic
Reaction Slope	:	Increasing.
Wavelength	:	340 nm
Flow cell temp	:	37°C

Delay time	:	60 secs.
No.of Readings	:	4
Interval	:	60 secs.
Sample Vol.	:	100 µl
Reagent Vol.	:	1.0 ml
Pathlength	:	1 cm
Factor	:	1746
Zero setting with	:	Distilled water

The reconstituted Reagent and sample are dispensed into the test tube, mixed and reading taken immediately.

Linearity :

The method is linear upto 260 u/L

Reference Values :

Serum / Plasma AST : Upto 46 U/L (at 37^0C)

ESTIMATION OF SERUM ALKALINE PHOSPHATASE

Method : Kinetic Method [49]

Principle :

In buffered alkaline medium alkaline Phosphatase hydrolyse P-Nitrophenol which produces a yellow colour. Intensity of the colour so produced is directly proportional to alkaline phosphatase activity and is measured photo metrically at 405 nm

Reagents :

Reagent 1 : DEA Buffer

Diethanolamine	1M
Magnesium Chloride	0.5 mM

Reagent 2 : PNPP Tablets

Para Nitro Phenyl Phosphate	10 mM

Reagent Reconstitution :

Each tablet in Reagent 2 is mixed with 2 ml of Reagent 1 mixed well, by gentle swirling to dissolve the tablet.

The reagent may be stored at $2^{o}C$ – $8^{o}C$ for 3 days in ambor coloured bottles.

Procedure :

The following parameters are set in the instrument.

Reaction Type	:	Kinetic
Reaction Slope	:	Increasing.
Wavelength	:	405 nm
Incubation time	:	60 secs.
Interval time	:	60 secs.
Interval No.	:	3
Factor	:	2713
Flow cell temp	:	37^{o}C
Molar extinction Coefficient	:	18.7
ΔOD /mt Limit	:	0.55
Units	:	1U/L
Upper normal Value	:	810 U/L
Lower normal Value	:	1101 U/L
Working Reagent Vol	:	1 ml
Sample Vol	:	100 µl
Reagent Vol	:	20 µl

The reconstituted reagent and the sample are dispensed into the test tubes, mixed well and after 1 minute incubation absorbance is read, photo metrically at 405 nm at 37^{o}C .

Linearity : Upto 1500 Iu/L

Reference Value :

Children	:	270 – 810 Iu/L
Adults	:	110 – 310 Iu/L

ESTIMATION OF SERUM SGPT

Method :

(Mod. IFCC method) For the determination of SGPT (ALT) activity in serum [49]

2. Principle :

SGPT (ALT) catalyzes the transfer of amino group between L-Alanine and α ketoglutarate to form Pyruvate and Glutamate. The Pyruvate formed reacts with NADH in the presence of Lactate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGPT (ALT) activity in the sample.

$$\text{L-Alanine} + \alpha\text{ ketoglutarate} \xrightarrow{\textbf{SGPT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\textbf{LDH}} \text{Lactate} + NAD^+$$

3. Reagents :

L1 :	Enzyme Reagent	20ml	60ml
L2 :	Starter Reagent	5ml	15ml

Reagent Preparation

Reagent are ready to use.

Working reagent :

For sample start assays a singly reagent is required. Pour the contents of 1 bottle of L2 (Starter Reagent) into 1 bottle of L1 (Enzyme Reagent). This working reagent is stable for at least 3 weeks when stored at 2-8^{o}C. Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of L1 (Enzyme Reagent) and 1 part of L2 (Starter Reagent). Alternatively 0.8ml of L1 and 0.2ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay.

Sample material

Serum : Free from hemolysis. SGPT (ALT) is reported to be stable in serum for 3 days at

2-8^{o}C.

4. Procedure :

Wavelength / filter	:	340 nm
Temperature	:	37^{o}C /30^{o}C/25^{o}C
Light path	:	1 cm

Substrate Start Assay :

Pippette into a clean dry test tube labeled as Test (T) :

Addition Sequence	**(T)** **25°C/30°C**	**(T)** **37°C**
Enzyme Reagent (L1) Sample	0.8 ml 02 ml	0.8ml 0.1ml
Incubate at the assay temperature for 1 minute and add		
Starter Reagent (L2)	0.2ml	0.2ml

Mix well and read the initial absorbance Ao & repeat the absorbance reading after every 1,2 & 3 minutes. Calculate the mean absorbance change per minute (Δ A/min.)

ESTIMATION OF SERUM UREA

1. UREASE METHOD

Intended use

This reagent kit is intended for in vitro quantitative determination of Urea in serum or plasma[49].

Clinical Significance.

Urea is the main end product of protein metabolism. Liver is the site of urea synthesis and urea is excreted by kidney.

Increases of serum or plasma urea concentration are associated with dehydration, shocks, fevers, acute glomerulonephritis and urine retention. Low serum or plasma urea levels in clinical diseases such as severe liver damage due to viral hepatitis are rare.

Principle.

This procedure is based on the Berthelot's reaction. Urease splits urea into ammonia and carbon dioxide. The ammonia reacts with phenol in presence of hypochlorite to form an indophenol which with alkali gives a blue coloured compound. The intensity of the colour is proportional to the concentration of urea

in the sample and is measured at 546nm (530-570nm). The colour of the reaction is stable for 8 hours.

Sample Collection, Storage & Stability

Serum is preferred, plasma can also be used. Anticoagulants such as heparin, Potassium oxalate, or EDTA can be used. Ammonium salts and fluoride should not be used. Serum or plasma urea determination should be carried out as far as possible on the same day. Samples are stable for a week when stored tightly capped at 2-8^{o}C or for a month at – 10^{o}C.

Do not use hemolysed or grossly contaminated samples.

3. Reagents

Reagent 1 (Urease) :

Urease	> 1 KSU/L

Reagent 1 A (Buffer) :

Disodium EDTA	0.1 mol/L
Sodium Nitroprusside	6 mmol/L

Reagent 2 (Phenol) :

Phenol	1.8 mmol/L

Reagent 3 (Hypochlorite) :

Sodium Hypochlorite	0.47 mol/L

Standard (Urea 40mg/dL) :

Urea 0.4 g/L

Reagent Reconstitution

Allow the reagents to attain room temperature.

Solution (1)

Transfer the contents of one bottle of reagent 1A into one bottle of reagent 1. Mix gently.

Solution(2)

Add 77ml of distilled water into one bottle of reagent 2. Mix gently.

Solution (3)

Add 77mL of distilled water into one bottle of reagent 3. Mix gently.

RECONSTITUTED REAGENT STORAGE & STABILITY

When stored at a2-8^{o}C, the reconstituted solutions 1,2,&3 are stable for 4 months.

Procedure :

The samples and the reconstituted solutions should be brought to the room temperature prior to use.

The following general system parameters are to be used with this kit.

General System Parameters

Reaction Type	:	Endpoint
Reaction Slope	:	Increasing
Wavelength	:	546 nm (530-570 nm)
Flowcell Temp.	:	30^{o}C
Incubation	:	10 Min. (1st step) & 15 min (2nd step) at 37^{o}C
Sample Vol.	:	10 µL
Reagent Vol.	:	3.1 mL(Reagents 1+2+3)
Std. Concentration	:	40mg/dL
Zero setting with	:	Reagent Blank

Set the instrument using above system parameters.

Dispense into test tubes:

	Blank	**Standard**	**Test**
Solution 1	100µ L	100µ L	100µ L
Standard	--	10µL	--
Sample	--	--	10µL

Incubate for 10 min. at 37^{o}C. Mix and then add:

Solution 2	1.5 mL	1.5mL	1.5 mL
Solution 3	1.5 mL	1.5mL	1.5 mL

Incubate for 15 min. at 37^{o}C. Mix and read.

LINEARITY

The method is linear up to 200mg/dL. For high values, dilute the sample suitably with 0.9% saline and repeat the assay. Apply proper dilution factor to calculate the final result.

REFERENCE VALUES

It is recommended that each laboratory establish its own reference values. The following values may be used as a guideline:

UREA :

Serum / Plasma : 10-50 mg/dL

ESTIMATION OF SERUM CREATININE

1. **Method** : Picrate Method[49] CODE 746 1x50 mL
   747 2x50 mL
   748 4x50 mL

2. **Principle**

Creatinine in alkaline solution reacts with picrate to form red-orange compound. Under the specific conditions of the assay, the rate of development of the colour is proportional to the concentration of creatinine in the sample when measured at 500 nm (490-510nm).

3. **Reagents**

Reagent a (Picrate) :

Picric Acid	34.9 mmol/L
Sodium Hydroxide	45 mmol/L

Reagent 2 (Sodium Hydroxide) :

Sodium Hydroxide	0.26 mol/L

Standard (Creatinine 2 mg/dL):

Creatinine	0.020 g/L

4. Preparation of working solution

Allow the reagents to attain room temperature. Mix equal volumes of reagent 1 & reagent 2 in a clean beaker.

5. Procedure

The samples and working solution should be brought to room temperature prior to use.

The following general system parameters are to be used with this kit:

General System Parameters

Reaction Type	:	Fixed Time
Reaction Slope	:	Increasing
Wavelength	:	500 nm (490-510nm)
Flowcell Temp.	:	25^{o}C, 30^{o}C or 37^{o}C
Delay Time	:	30 secs.

No.of Readings	:	2
Interval	:	120 Secs
Sample Vol	:	100µ L
Reagent Vol.	:	1.0 mL
Path length	:	1 cm
Std. Concentration	:	2 mg/dL
Zero Setting with	:	Distilled Water

Set the instrument using above system parameters.

1. CALIBRATION

Dispense into test tube :

	Standard
Working Solution	1 mL
Standard	100µL

Mix and read immediately for factor calculation.

2. TEST

Dispense into test tube:

	Standard
Working Solution	1 mL
Standard	100µL

Mix and read immediately.

LINEARITY

The method is linear up to 10 mg/dL. For higher values, dilute the sample suitably with .9% saline and repeat the assay. Apply proper dilution factor to calculate the final result.

REFERENCE VALUES :

It is recommended that each laboratory establish its own reference values. The following values may be used as a guideline :

Creatinine :

Serum / Plasma

Females : 0.5 - 0.9 mg/dL

RESULTS

S.No.	Gamma Glutamyl Transaferase (GGT)	FBS Fasting Blood Sugar	HDLC	TGL	TOTAL CHOLES-TEROL	URIC ACID	SGOT	SGPT	ALK. PHOSPH.	UREA	CREA-TININE	LDL	AGE	BMI	B.P.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
1	12.5	103	**48**	**207**	**259**	**10.3**	34.2	31	163.1	29	0.9	**169.6**	32	**36.6**	**150/110**
2	**26.3**	**111**	**37.6**	**179**	**255**	6.9	31.5	24.4	227	21	0.6	**181.2**	45	28.5	**140/90**
3	**29.2**	**112**	**35**	**225**	**237**	**9.7**	39.1	23.2	171	14	0.6	**207**	40	**32.6**	**170/100**
4	**28.00**	102	**40.4**	**206**	**308**	**7.8**	39.7	30.3	209	25	0.8	**225**	48	**34.6**	134/90
5	15	85	**39.7**	**206**	**227**	4.9	28.2	19.3	124	19	0.9	146.1	42	**31.2**	**140/90**
6	**29.9**	92	**42**	**190**	**235**	6.8	36.3	24.3	172	22	0.9	155	52	**30.8**	**136/100**
7	**33.2**	108	**38.6**	**337**	**212**	**8.2**	34.9	23.9	169	21	0.9	106.4	55	**30.5**	**150/90**
8	13	84	**43**	140	**235**	4.4	29.8	18.00	184	21	0.7	164	45	29.56	**134/100**
9	**27.2**	**112**	**33.7**	**287**	**288**	4.2	36.8	26.2	204	24	0.8	**196.5**	50	**37.17**	**150/100**
10	**30.7**	**137**	**29.1**	**231**	219	**10.6**	32.5	22.9	112	28	1	144	65	**37.4**	140/80
11	21.7	72	**31.7**	**214**	217	6.2	35.8	26.7	210	32	0.9	108	52	**37.7**	**150/90**
12	**30.1**	108	**38.6**	**202**	186	6.6	26.7	18.6	147	20	0.9	152	55	**35.4**	**136/90**
13	**27.01**	103	**30**	**201**	**244**	3.4	29.6	20.8	216	17	1.1	174	50	**31.3**	**150/90**
14	**25.5**	85	**37.6**	**245**	219	5.8	29.2	24.3	216	15	0.8	132	37	**32.7**	**180/110**
15	**27.76**	85	**30.2**	**195**	210	6.7	29.3	26.6	161	21	1	141	65	**35.12**	**150/90**
16	19.72	79	**42.6**	**186**	207	**9.2**	30.6	23.4	164	30	0.7	127.4	65	**33.78**	**150/90**
17	5.93	82	**44.1**	**169**	174	5.3	36.7	13.6	150	16	0.7	78	38	27.75	136/90
18	**25.58**	75	**38.2**	**186**	**220**	4.3	27.3	18.6	143	19	0.9	111	65	**30.1**	**140/90**
19	12.6	94	**44.3**	**196**	193	4.8	32.1	28.6	157	19	0.8	110	47	29.52	136/90
20	**28.2**	84	**42.3**	**183**	**226**	5.1	31.6	28.4	156	30	0.8	142.6	50	**30.1**	**160/100**

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
21	29.6	101	**38.4**	**187**	204	6.2	29.3	27.6	147	16	0.8	127.8	55	**32.90**	**140/90**
22	**27.3**	**112**	**37.6**	**179**	196	5.8	31.4	26.7	147	21	0.9	123	60	29.82	**150/90**
23	18.1	95	**42.3**	**182**	167	4.3	38.67	30.1	212	18	0.7	88.6	55	30	**150/100**
24	**27.59**	100	**33.7**	**177**	208	6.4	34.3	21.6	126	22	0.9	169	60	**30.22**	130/90
25	23	92	**40**	**204**	193	5.2	37.1	30.9	162	17	0.6	112	58	27.62	**140/90**
26	19.6	101	**40.4**	**178**	166	**7.8**	30.5	26.5	204	21	0.7	90	60	29.57	**140/90**
27	**37.3**	**123**	**36.3**	**239**	**251**	**8.8**	29.3	29.1	156	28.5	1	166.9	72	**34.28**	136/90
28	**26.3**	105	**39.3**	**193**	210	**7.6**	30.3	28.6	168	16	0.9	132.1	51	29.61	**140/90**
29	34.3	76	**40.2**	**192**	196	6.0	39.1	18.9	155	17	0.9	128	57	**32.00**	**140/100**
30	**26.5**	100	**32.4**	**176**	213	4.6	38	28.6	210	14	0.8	145.6	40	**32.07**	**140/100**
31	**27.1**	101	**40.6**	**189**	201	5.8	42.1	28.4	169	19	1	132	60	29.58	**150/100**
32	18.6	102	**32.4**	**201**	188	5.5	38.9	19.3	191	25	0.6	115.4	45	**30.3**	**140/96**
33	**32.5**	105	**28.3**	**190**	198	5.8	40.1	23.4	148	15	0.8	172	62	**43.55**	**140/96**
34	18.3	98	**35.6**	**184**	200	6.6	29.7	18.1	136	13	0.9	128	42	**32.17**	**140/90**
35	**28.9**	102	**46.1**	**179**	203	5.8	29.3	19.8	192	19	0.7	142	49	**35.00**	136/90
36	**36.7**	108	**39.5**	**206**	217	6.1	40.2	19.6	211	27	0.9	150	68	**30.08**	**140/90**
37	**34.2**	106	**37.8**	**213**	**241**	**7.3**	32.1	19.6	169	18	0.6	160	50	**33.84**	**140/100**
38	**38.9**	106	**33.6**	**246**	210	**7.3**	37.1	23.6	193	28.1	0.9	146	40	**37.22**	136/90
39	**26.2**	96	**39.1**	**198**	207	**7.5**	31.6	24.1	166	27	0.8	138	52	29.82	**150/100**
40	**27.2**	109	**39.3**	**183**	**237**	**7.6**	40.1	26.6	172	20	0.9	160	60	**31.53**	**140/94**
41	14.7	99	**41.3**	**177**	193	4.9	34.6	29.1	177	20.6	0.8	118	45	**30.66**	136/90
42	**40.2**	94	**33.4**	**208**	**242**	**7.6**	39.6	23.9	211	28	0.9	168	60	29.56	**140/94**
43	22.3	103	**39.4**	**200**	230	**8.7**	29.6	19.2	159	22	0.8	136.2	56	**32.52**	**180/100**
44	16.7	104	**42.6**	**184**	191	6.1	43.2	28.7	171	25.4	0.9	112	48	**42.57**	**140/96**

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
45	10.7	87	**44.3**	**188**	200	4.9	40.3	27.4	147	20.6	0.7	121.4	39	29.91	**140/90**
46	29	106	**40.2**	**196**	238	6.6	33.6	28.7	193	18	0.7	148	42	**30.76**	**150/100**
47	**26.8**	98	**38.9**	**207**	236	6.5	40.3	29.6	206	19.7	0.8	165	54	30.00	**140/96**
48	**38.3**	103	**36.2**	**224**	239	6.8	35.8	23.1	241	30	1	188	46	**30.75**	**140/94**
49	**25.3**	**110**	**34.2**	**219**	208	6	32.9	29.7	243	20	0.8	136	64	30.04	**140/94**
50	**26.68**	94	**43.1**	**197**	240	6.8	31.6	29.3	184	15	0.8	157.2	47	**31.62**	**136/90**
51	16.5	98	**40.3**	**196**	180	6.1	37.9	18.6	199	32	0.9	98.1	40	**31.43**	**140/90**
52	23.6	109	**38.9**	**188**	221	6.3	41.6	25	193	26.3	1	144.5	60	**34.34**	**140/100**
53	**32.6**	102	**39.1**	**203**	226	6.2	43.2	29.8	166	25	0.9	146.3	64	30.6	**160/90**
54	21.3	105	**39**	**200**	197	5.9	39.7	21.6	211	20	0.9	118	52	**30.90**	**150/96**
55	18.1	86	**43.9**	**185**	190	5.1	36.7	24.6	160	20	1	109.1	53	29.90	136/90
56	19.97	101	**42..4**	**180**	202	6.6	44	27.1	20	24.1	0.8	123.6	45	30.90	**140/94**
57	20.1	102	**36.9**	**216**	201	5.8	41.3	18.4	198	23	0.8	125.8	42	**32.50**	136/96
58	**30.3**	109	**39.7**	**248**	242	**8.7**	34.3	27.6	210	23	1	152.7	65	**32.26**	**140/90**
59	9.7	96	**44.6**	**179**	196	4.3	37.9	21.9	129	18	0.6	115.6	37	**31.35**	**146/90**
60	7.37	89	**48.6**	**180**	186	4.8	32.2	18.8	171	35	0.8	101.4	42	**35.82**	**136/100**
61	18.1	101	**46**	**192**	186	7.3	39	27.3	196	18.6	0.8	99	48	30	136/90
62	**25.6**	100	**40.6**	**188**	222	**9.3**	38	25.9	181	19.6	0.9	143.8	50	**31.5**	136/96
63	21.8	97	**43.6**	**189**	200	6.1	39.8	23.6	194	19.9	0.9	128.8	50	**33.1**	**150/96**
64	**28.1**	110	**40.1**	**215**	255	7	38	26.7	147	20	0.9	172	52	**32.8**	**170/100**
65	20.9	101	**40**	**196**	204	5.5	39.4	28	149	19.8	0.8	124.8	48	30.4	**140/90**
66	**25.9**	104	**39.6**	**194**	222	6.2	34.3	26.1	182	20	0.8	143.6	44	**35.4**	**140/96**
67	22.1	102	**43**	**186**	194	5.4	39.7	23.4	169	19	0.9	123.8	50	30	**146/96**
68	17.6	84	**44.6**	**179**	206	6.9	34.7	31	127	16	0.7	125.6	46	**30.02**	**140/90**
69	**29**	106	**37.9**	**210**	240	6.4	42.6	29.6	210	26	1	160.1	60	**31.4**	**150/100**

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
70	**31.8**	107	**40.6**	**204**	222	**8.4**	34.9	29.1	196	32	1	140	55	**32.91**	**150/100**
71	**43.3**	100	**35.3**	**198**	261	**10.7**	33.5	28.6	193	27	0.7	186.1	53	**36.66**	**150/100**
72	**25.6**	89	**38.6**	**192**	207	6.6	43.1	26.7	196	21	0.7	134	57	**30.22**	**140/96**
73	14.9	103	**42.6**	**177**	191	6.2	42.6	30.1	181	16	0.7	113	51	29.62	**140/90**
74	20.9	98	**45.1**	**177**	193	7	42.6	23.9	147	20	0.8	122.5	43	**31.11**	136/86
75	**27.36**	89	**30.76**	**198**	232	**9.1**	34.9	20.8	216	24.6	1	161.8	61	**32.10**	**160/100**
76	**40.3**	107	**39.7**	**261**	243	**7.3**	41.9	27.6	193	30.1	0.9	152	49	**34.16**	**150/100**
77	24.7	108	**40.5**	**193**	204	6.3	39.1	31	151	28	0.9	140	65	**30.47**	140/96
78	18.9	104	**42**	**190**	202	5.8	36.3	24.1	158	21.6	0.7	122	43	**30.86**	**140/100**
79	**28.3**	108	**42.1**	**196**	206	**8.6**	36.7	29.8	184	20	0.9	146.4	55	**31**	**170/100**
80	**27.6**	103	**38.9**	**198**	216	**7.1**	38.1	29.1	149	30.1	1	142.5	58	**34.28**	**160/100**
81	17.94	92	**44.6**	**189**	194	6.7	41.9	29.4	206	23.6	0.9	116	56	30.01	**150/90**
82	23.3	84	**42.2**	**201**	212	4.7	30.7	27.6	193	17.9	0.7	134	39	**31**	136/90
83	15.6	98	**41.9**	**179**	190	4.3	37.9	28.1	210	18.6	0.6	112.3	51	30	130/90
84	20.5	96	**44**	**178**	200	4.1	42.1	26.6	170	16.5	0.7	120.4	41	30.02	136/90
85	20.1	108	**44.5**	**212**	195	5.9	40.6	20.4	191	27	0.9	138	40	**33.33**	136/96
86	19.6	94	**35.7**	**197**	222	6.4	38	24.6	179	29	0.8	127	65	**32.03**	**154/100**

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
87	**29.1**	102	**40.9**	**204**	219	6.3	43.1	20.6	203	28	0.9	168	62	30.26	**150/90**
88	26.4	100	**42**	**199**	244	6.8	36.1	21.9	173	20.3	0.7	163.4	40	**33.30**	**140/96**
89	28.9	96	**37.5**	**259**	227	**7.8**	31.7	19.6	149	28	0.8	170	55	**34.75**	**160/94**
90	18.7	86	**49**	**180**	190	6.1	34.6	18.1	173	30	0.8	98	52	**31.5**	**170/90**
91	21.6	103	**42.1**	**197**	206	5.8	36.6	29.9	167	21	0.9	126.5	42	**34.66**	**150/90**
92	**30.1**	106	**40**	**204**	237	6.8	37.6	29.8	112	24	0.8	156.2	48	**40**	**140/100**
93	23.6	104	**41.7**	**200**	206	**7.1**	42.6	21.9	143	25	0.8	136.3	51	**34**	**146/90**
94	**26.8**	96	**39**	**217**	224	7	42	28.8	161	22.1	1	15.6	65	**33.91**	**150/100**
95	17.9	101	**44.1**	**179**	196	6.7	42	28.9	182	22.4	0.8	117	57	30	**140/94**
96	**26.2**	103	**39.4**	**196**	231	6.1	40.7	27.6	201	19.6	0.8	152.8	49	**34.27**	**150/100**
97	**36.2**	106	**40**	**230**	245	**10.1**	32.9	26.7	191	30.1	1	159	56	**34.63**	**140/100**
98	18.1	98	**42**	**200**	187	5.8	38.7	27.3	194	19	0.9	105	40	**30.47**	**140/96**
99	17.8	80	**46**	**186**	192	4.9	39.6	18.9	171	16	0.8	109	42	**30.90**	136/90
100	**35.4**	101	**38.7**	**201**	247	6.6	40.6	30.1	147	21	0.9	158.3	58	30	**140/94**

Table : 1

DISTRIBUTION OF VARIABLE CLASSES IN THE POPULATION STUDIED

GGT		AGE		FBS		URIC ACID		HDLC		TOTAL CHOLESTROL	
Class	**Frq.**	**Class**	**Frq.**	**Class**	**Frq.**	**Class**	**Frq.**	**Class**	**Frq.**	**Class**	**Frq.**
4.5-9.9	2	30-34	1	70-79	4	3.0-3.9	1	28.5-30.9	5	160-179	3
10.0-14.9	7	35-39	5	80-84	6	4.0-4.9	15	31.0-33.4	4	180-199	25
15.0-19.9	19	40-44	18	85-89	9	5.0-5.9	18	33.5-35.9	8	200-219	33
20.0-24.9	17	45-49	17	90-94	7	6.0-6.9	36	36.0-38.4	11	220-239	22
25.0-29.9	36	50-54	20	95-99	14	7.0-7.9	16	38.5-40.9	35	240-259	14
30.0-34.9	10	55-59	16	100-104	32	8.0-8.9	6	41.0-43.4	19	260-279	1
35.0-39.0	6	60-64	13	105-109	20	9.0-9.9	4	43.5-45.9	12	280-300	1
40.0-45.0	3	65-70	10	110-115	8	10.0-10.9	4	46.0-48.5	6	300-320	1

TGL		LDL		BMI		CREATININE	
Class	**Frq.**	**Class**	**Frq.**	**Class**	**Frq.**	**Class**	**Frq.**
149-169	2	60-79	1	24.0-26.4	1	0.6	7
170-184	22	80-99	5	26.5-28.9	2	0.7	16
185-199	36	100-119	18	29.0-31.4	50	0.8	29
200-214	22	120-139	27	31.5-33.9	22	0.9	33
215-229	7	140-159	27	34.0-36.4	15	1.0	14
230-244	3	160-179	16	36.5-38.9	7	1.1	1
245-259	4	180-199	4	39.0-41.4	1		
260-275	4	200-219	2	41.5	2		

Variability for characters in the Population Studied

Table : 2

S.No.	*Character*	*Population Size*	*Range*		**Mean**	*Standard Error (Mean)*	*Standard Deviation*	*Coefficient of variation (CV%)*
			Min.	**Maxi.**				
1	GGT	100	5.9	43.3	24.64	0.74	7.38	30.00
2	FBS	100	72.0	137.0	99.21	1.01	10.06	10.14
3	HDLC	100	28.3	49.0	39.74	0.42	4.20	10.57
4	TGL	100	140.0	337.0	199.97	2.53	25.30	12.65
5	T.Chl.	100	166.0	308.0	215.05	2.44	24.44	11.36
6	Uric Acid	100	3.4	10.7	6.53	0.15	1.49	22.77
7	SGOT	100	26.7	44.0	36.41	0.45	4.50	12.35
8	SGPT	100	13.6	31.0	25.16	0.41	4.10	16.31
9	Alk. Phosp.	100	112.0	243.0	176.98	2.75	27.51	15.59
10	Urea	100	13.0	35.0	22.19	0.49	4.93	22.21
11	Creatin	100	0.6	1.1	0.83	0.01	0.12	13.76
12	LDL	100	78.0	225.0	138.87	2.63	26.30	18.90
13	Age	100	32.0	72.0	51.42	0.86	8.59	16.70
14	BMI	100	25.5	43.6	32.20	0.30	2.95	9.16
15	B.P.(S)	100	130.0	180.0	144.54	1.00	10.01	6.92
16	B.P.(D)	100	86.0	110.0	94.44	0.50	4.95	5.24

Table : 3 Partial Correlations – Exclusion of variables.

Model No	*Variable*	*Beta' in*	*'t'*	*Significant Level*	*Partial correlation (PC)*
1	FBS	0.176 [a]	2.095	0.039*	0.208
	T.Chol	0.021 [a]	0.132	0.895[NS]	0.013
	TGL	0.332 [a]	4.334	0.006**	0.403
	Creatin	0.246 [a]	3.197	0.002**	0.309
	BMI	0.066 [a]	0.792	0.430[NS]	0.080
	Uric Acid	0.251 [a]	3.048	0.003**	0.296
	Age	0.376 [a]	5.301	0.000**	0.474
2	FBS	0.120 [b]	1.584	0.116 [NS]	0.160
	T.Chol	0.047 [b]	0.342	0.733 [NS]	0.035
	TGL	0.300 [b]	4.425	0.000*	0.412
	Creatin	0.166 [b]	1.515	0.133 [NS]	0.153
	BMI	0.068 [b]	0.926	0.357 [NS]	0.094
	Uric Acid	0.164 [b]	2.136	0.036*	0.212
3.	FBS	0.044	0.604	0.547 [NS]	0.062
	T.Chol	0.132	-0.983	0.323 [NS]	0.101
	Creatin	0.053	0.733	0.465 [NS]	0.075
	BMI	0.007	0.100	0.920 [NS]	0.010
	Uric Acid	0.097	1.330	0187 [NS]	0.135

Influenced variable : GGT

a. Predictors in Model 1 : (Constant), LDL

b. Predictors in Model 2 : (Constant), LDL, Age

c. Predictors in Model 3 : (Constant), LDL, Age, TGL

Table : 4 Correlation (r) among variables (Pearson's)

	FBS	HDLC	TGL	T.CL.	Uric Acid	SGOT	SGPT	Alk. Pho.	Urea	Creatin	LDL	Age	BMI	BPS	BPD
GGT	0.383**	-0.528**	0.487**	0.538**	0.449**	-0.051	0.134	0.151	0.193	0.338**	0.618**	0.449*	0.238*	0.146	0.144
FBS		-0.270**	0.346**	0.259**	0.380**	0.116	0.212*	0.045	0.113	0.194	0.374**	0.182	0.212*	-0.001	0.041
HDL			-0.345**	-0.308**	-0.167	0.160	0.062	-0.127	-0.022	-0.246*	-0.423**	-0.363**	-0.261**	-0.107	-0.038
TGL				0.400**	0.323**	-0.047	0.042	0.133	0.242*	0.260**	0.298**	0.122	0.273**	0.253*	0.118
T.Chl.					0.375**	-0.110	0.194	0.156	0.203*	0.162	0.861**	0.092	0.231*	0.179	0.309**
Uric Acid						-0.092	0.142	-0.031	0.334	0.208*	0.378**	0.270**	0.263**	0.243*	0.176
SGOT							0.237*	0.168	0.072	0.022	-0.057	-0.005	-0.010	-0.061	0.086
SGPT								0.099	-0.023	0.055	0.100	0.157	0.020	0.026	0.116
Alk. Pho									0.135	0.015	0.127	-0.022	-0.171	0.035	0.180
Urea										0.335**	0.122	0.248*	0.280**	0.130	0.148
Crea-tin											0.159	0.402**	0.221	0.110	0.044
LDL												0.129	0.288**	0.130	0.277**
Age													0.032	0.111	-0.111
BMI														0.085	0.242*
BPS															0.441**

BPD

N = Population size = 100

* = Correlation Significant at 0.05 level

** = Correlation significant at 0.01 level.

Table : 5

χ^2 Test for independence between GGT and other variables (Summary)

S.No.	Grouping based on Clinically normal levels					Grouping based on sturges rule with combination of marginal classes			
	Variables	d.f	χ^2	'P' level	Coeff. Of Mean square contingency	d.f	χ^2	'P' level	Coeff. Of Mean square contingency
1.	GGT-FBS	1	8.09	0.005	0.274	4	12.82	0.020	0.337
2	GGT-TGL	1	5.06	0.025	0.219	4	19.26	0.001	0.402
3	GGT-T.Chol.	1	31.87	0.0001	0.492	4	38.48	<0.001	0.527
4	GGT-Uric Acid	1	10.71	0.001	0.311	4	25.68	<0.001	0.452
5	GGT-Creatinine	1	5.73	0.025	0.233	4	10.67	0.050	0.311
6	GGT-Age	1	7.20	0.010	0.259	3	12.33	0.025	0.331
7	GGT-BMI	1	3.90	0.05	0.194	6	17.12	0.010	0.382
8	GGT-LDL	1	48.54	0.0001	0.572	6	48.18	<0.001	0.570
9	GGT-HDLC	1	23.71	0.0001	0.438	4	13.33	0.010	0.343

N = 100

STATISTICAL ANALYSIS

Table – 6 (a)

Age and GGT Positivity

AGE / GGT	30-39 Yrs.	40-49 Yrs.	50-59 Yrs	≥ 60 Yrs.	TOTAL
15-25	5	21	14	5	45
≥ 25	1	14	22	18	55
TOTAL	6	35	36	23	100

DF = 3

χ^2 = 12.33

Significant at level of P=0.025
coefficient of mean square contingency = 0.331

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Table – 6 (b)

Age and GGT Positivity

AGE / GGT	≤ 44 yrs	≥ 45 Yrs	TOTAL
≤ 25	17	28	45
> 25	7	48	55
TOTAL	24	76	100

DF = (2 – 1) (2-1)
= 1 = 1

χ^2 = 7.20

Significant of P= 0.010 level
Coefficient of mean square contingency = 0.259

Table – 7(a)

GGT and Fasting Blood Sugar levels

Fasting Blood Sugar / GGT	70-90 mg/dl	91-100 mg/dl	100-120 mg/dl	TOTAL
Upto 15	2	5	2	9
15-25	5	14	17	36
≥ 25	3	11	41	55
	10	30	60	100

D.F = (3-1) (3-1) 2x2 = 4
χ^2 = 12.82
Significant at P= 0.020 level
CMSC = 0.337

Table – 7(b)

GGT and Fasting Blood Sugar levels

Fasting Blood Sugar / GGT	≤106 mg/dl	≥107 mg/dl	TOTAL
≥ 25	42	3	45
>25	39	16	55
TOTAL	81	19	100

D.F = (2-1) (2-1) = 1x1=1

χ^2 = 8.09

Significant at P= 0.005 level

Table – 8(a)

GGT and Uric acid levels

Uric Acid / GGT	<5mg/dl	5-7 mg/dl	>7mg/dl	TOTAL
<15	6	2	1	9
15-25	6	24	6	36
> 25	4	28	23	55
TOTAL	16	54	30	100

D.F = 4

χ^2 = 25.68

Significant at P< 0.001 level

CMSC = 0.452

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Table –8(b)

GGT and Fasting Uric Acid levels

Uric Acid / GGT	≤ 6 mg/dl	>6 mg/dl	TOTAL
≤ 25	24	21	45
≥ 25	11	44	55
TOTAL	35	65	100

D.F = 1

χ^2 = 10.71

Significant at P= 0.001 level

Coefficient of mean square contingency = 0.311

Table – 9(a)

GGT and Low Density Lipoprotein

LDL / GGT	<5mg/dl	5-7 mg/dl	>7mg/dl	TOTAL
≤15	6	1	2	9
15-25	29	6	1	36
25-35	5	25	16	46
35-45	1	4	4	9
TOTAL	41	36	23	100

D.F = (4-1) x (3-1) = 3x2=6

χ^2 = 48.18

Significant at P< 0.001 level

CMSC = 0.570.

Table – 9(b)

GGT and Low Density Lipoprotein levels

LDL / GGT	≤ 130 mg/dl	>130 mg/dl	TOTAL
≤ 25	36	9	45
> 25	5	50	55
TOTAL	41	59	100

D.F = 1

χ^2 = 48.54

Significant at P= 0.0001 level

Coefficient of mean square contingency = 0.572

Table – 10(a)

GGT and High Density Lipoprotein

HDL-C / GGT	≤33.5	33.6 – 38.4	≥38.5	TOTAL
≤25	2	3	40	45
25-35	6	12	28	46
35-45	1	4	4	9
TOTAL	9	19	72	100

D.F = 4

χ^2 = 13.33

Significant at P= 0.010 level

CMSC = 0.343

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Table– 10 (b)

GGT and High Density Lipoprotein levels

All within the normal level, Hence population mean level was taken as the point of division for HDLC

HDL-'C' / GGT	≤ 39.72mg/dl	>39.73mg/dl	TOTAL
≤25	9	36	45
> 25	39	16	55
TOTAL	48	52	100

D.F = 1
χ^2 = 23.71

Significant at P= 0.0001 level
Coefficient of mean square contingency = 0.438

Table – 11(a)

GGT and Total Cholesterol levels

Cholesterol / GGT	160-200 mg/dl	200-240 mg/dl	>240mg/dl	TOTAL
≤15	7	1	1	9
15-25	21	14	1	36
> 25	4	35	16	55
TOTAL	32	50	18	100

D.F = 4

χ^2 = 38.48

Significant at P<0.001 level

CMSC = 0.527

Table 11 (b)

GGT and Total Cholesterol levels

Total Cholesterol / GGT	150-200 mg/dl	≥201 mg/dl	TOTAL
≤ 25	28	17	45
≥ 25	4	51	55
TOTAL	32	68	100

D.F = 1

χ^2 = 31.87

Significant at P= 0.0001 level

Coefficient of mean square contingency = 0.492

Table – 12(a)

GGT and Triglyceride level :

Triglyceride / GGT	≤185mg/dl	185-215 mg/dl	216-245 mg/dl	TOTAL
≤15	6	2	1	9
>15-25	11	23	2	36
> 25-45	70	32	16	55
TOTAL	24	57	19	100

D.F = 4
χ^2 = 19.26

Significant at P= 0.001 level
CMSC = 0.402

Table – 12(b)

GGT and Triclycerides levels

Population mean taken of the point of class separation for TGL

TGL / GGT	≤ 199.97mg/dl	≥199.98 mg/dl	TOTAL
≤ 25	33	12	45
> 25	27	28	55
TOTAL	60	40	100

D.F = 1
χ^2 = 5.06

Significant at P= 0.025 level
CMSC = 0.219

Table – 13(a)

GGT and Serum Creatinine level :

Creatinine / **GGT**	**≤0.7mg/dl**	**0.8-0.9 mg/dl**	**1-1.1 mg/dl**	**TOTAL**
≤25	15	28	2	45
26-35	7	29	10	46
36-45	1	5	3	9
TOTAL	23	62	15	100

D.F = 4

χ^2 = 10.67

Significant at P= 0.050 level

CMSC = 0.311

Table – 13(b)

GGT and Creatinine levels

Creatinine / GGT	0.5-0.9 mg/dl	>1 mg/dl	TOTAL
< 25	43	2	45
> 25	42	13	55
TOTAL	85	15	100

D.F = 1

χ^2 = 5.73

Significant at P= 0.025 level

Coefficient of mean square contingency = 0.233

Table – 14 (a)

GGT and Serum Body Mass Index level :

BMI / GGT	≤ 29	30-34	≥ 35	TOTAL
5-15	5	2	2	9
15-25	9	22	5	36
25-35	5	28	13	46
35-45	2	2	5	9
TOTAL	21	54	25	100

D.F = (4-1) x (3-1) = 3x2 = 6

χ^2 = 17.12

Significant at P= 0.010 level

CMSC = 0.382

Table – 14(b)

GGT and Body Mass Index levels

BMI / GGT	≤ 30 mg/dl	>30 mg/dl	TOTAL
≤ 25	16	29	45
> 25	9	46	55
TOTAL	25	75	100

D.F = 1

χ^2 = 3.90

Significant at P= 0.05 level

Coefficient of mean square contingency = 0.194

DISCUSSION

Gamma – Glutamyl Transferase is a cell-surface protein contributing to exracellular catabolism of Glutathione[50], the main thiol antioxidant in humans. The enzyme is produced in many tissues but serum GGT is derived mainly from Liver[50].

GGT is carried primarily by lipoproteins and albumin [51]. Serum levels of GGT are determined by factors like body fat, plasma lipid/lipoproteins, glucose levels, alcohol intake etc.

In my study conducted among 100 females with features of metabolic syndrome, 55 percent of them showed an increase in Serum GGT levels, and 45 percent were within normal limits. It was found that serum GGT had a positive and strong association with,

a. Age
b. Low Density Lipoprotiens
c. Triglycerides
d. Total cholesterol
e. Body Mass Index
f. Fasting Blood Sugar
g. Uric Acid

Metabolic syndrome can be considered a coronary artery disease equivalent[52]. Multiple pathophysiological mechanism play a role in the increased risk of cardiovascular events in the metabolic syndrome. These mechasims include hypertension, dyslipidemia etc.

In the present study, which included female patients of three different religions – Hindus, Muslims and Christians, trends indicate differences among these groups for the important risk factor LDL. The mean values for LDL is highest among Muslims (152.82mg/dl) and least among Christians (125.60mg/dl). Among Hindus it was (137.80mg/dl) next to Muslims with very close association to the population mean of 138.87

The levels of HDL cholesterol were converse, highest among Christians (40.48), among Hindus it was (39.86) and the lowest among Muslims (38.26).

Atherogenic dyslipdemia is an integral component of the metabolic syndrome and is a major contributor to the cardiovascular risks. In these patients an abnormal lipid profile is a more significant risk factor than either hypertension (or) diabetes mellitus alone. The typical lipid abnormalities defined in patients with metabolic syndrome consists of a triad:

1. Increased LDL Cholesterol
2. Increased Triglycerides
3. Decreased HDL Cholesterol

The small dense LDL particles are more atherogenic because they are more susceptible to oxidation[53]. The formation of early lesions of atheroscelerosis most often arise from focal increases in content of lipoprotein within regions of intima of arteries because they bind to constituents of extracellular matrix increasing the residence time of lipid rich particles with arterial wall. Lipoproteins which accumulate in the extracellular space of intima of arteries associate with proteoglycon of arterial extra cellular matrix and become susceptible to oxidative modification.

In our study, LDL showed the highest positive correlation with GGT at a level of (P=0.0001). 59 percent showed serum LDL levels greater than 130mg/dl. Similarly, age of the patients included in the study ranged from thirty two to seventy two years and showed positive correlation with GGT at a significant level of (P=0.001). Study results revealed increased levels of GGT with increase in age groups especially between forty to sixty years. But in Muslims the average age group was much lower and the risk set at an younger age compared to Hindus and Christians.

Similarly, GGT levels showed a strong positive correlation with serum triglycerides significantl at a 'P' value of (0.001). The mean triglycerides levels were highest among Muslims (217mg/dl), followed by Hindus (198.21mg/dl) and

(187.44mg/dl) among the Christians, probably due to varied intake of non-vegetarian diet.

Insulin normally suppresses the Production of VLDL particles from Liver. This effect is due to increase in free-fatty acid availability following Insulin inhibition of lipolysis in adipose tissue, and a direct hepatic effect of Insulin, inhibiting the production of VLDL particles. The intrahepatic defect appears to major contributory mechanisms underlying the increase in serum triglycerides in insulin resistance condition.

HDL levels are reduced in Insulin resistance patients[54] with high serum triglycerides. Under hypertriglyceridemic conditions there is excessive exchange of cholesterol esters and triglycerides between HDL and expanded pool of triglyceride rich lipoproteins mediated by the cholesterol ester transfer protein (CETP). HDL becomes enriched with triglycerides and acts as a good substrate for hepatic lipase which removes HDL at an accelerated rate. In my study, Pearson's method of correlation indicated negative correlation of HDL-C and GGT at (-0.528), which is highly significant negative correlation.

The mean body mass index (BMI) in my study was (32.20), with Hindus (31.95) Christians (32.04) and Muslims (33.69). Again body mass index is highest among the Muslim women and are more prone for increased GGT levels and hence coronary heart disease. Though, 50 percent of the females showed BMI

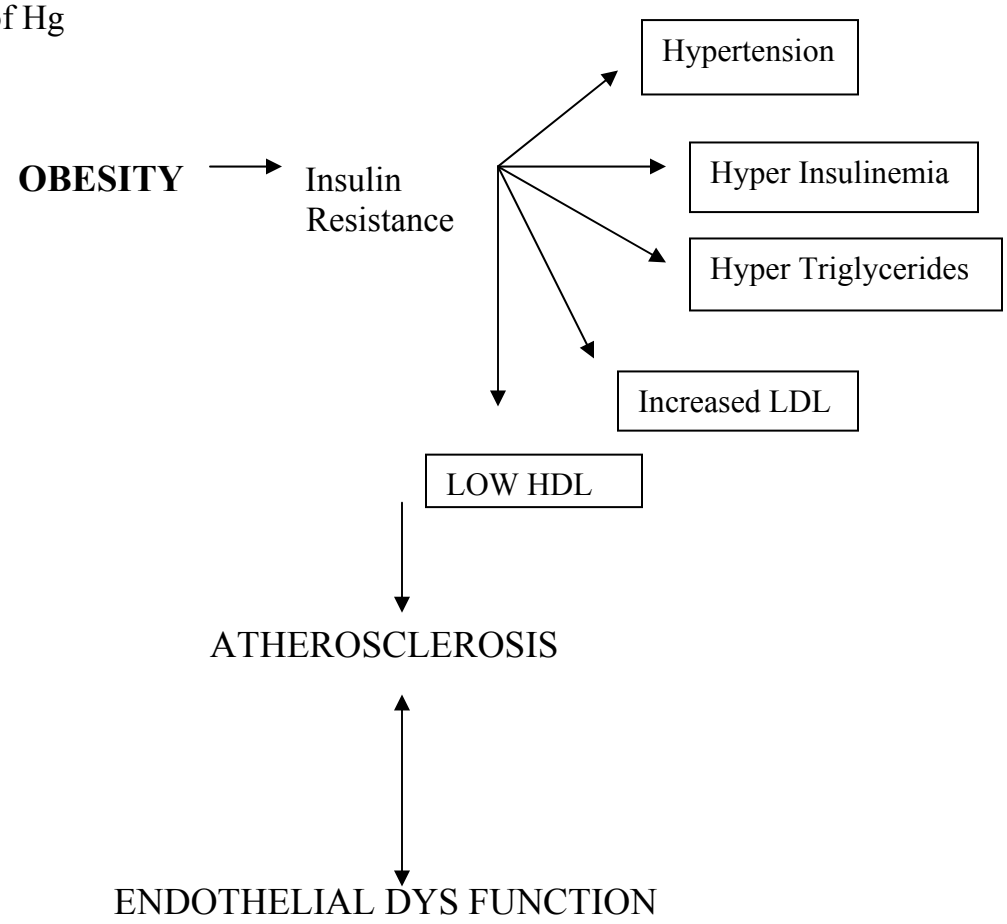
values between (29-31.4), 2 percent showed very high values of BMI about (41.50). GGT and BMI were significant with positive correlation at (P=0.05) level.

In obesity particularly visceral (or) central, adipocytes secrete number of biologicalproducts like Tumor Necrosis factor – alpha, free fatty acids, adiponectin, leptin and interleukin-6 that modulate insulin secretion, insulin action, body weight and contribute to insulin resistance. These biological substances secreted by adipocytes increase the amount of inflammation which can cause build up of plagues in vesselwalls. Eventually pieces of clots can break up and block blood vessels leading to myocardial infarction.

Persons with metabolic syndrome have a three fold greaser risk of coronary heart disease and four fold risk of cardiovascular mortality. The growth in prevalence of metabolic syndrome parallels the dramatic rise in Prevalence of obesity.[55,56]

In my study about 33 percent shown serum cholesterol levels between 200-219mg/dl and 25 percent showed 180-199mg/dl. 3 percent showed levels greater than 260mg/dl. The mean fasting blood sugar was 100-104mg/dl in 32 percent, 105-109mg/dl in 20 percent and 110-115mg/dl in 8 percent and 70-99mg/dl in 40 percent. GGT was positively correlating with fasting blood sugar at (P=0.0005) level.

The mean systolic blood pressure was 144.54 mm.Hg, ranging from maximum of 180mm.Hg and minimum of 130mm.Hg. The mean diastolic blood pressure was 94.4mm of Hg with a maximum of 110mm of Hg to a minimum 86 mm.of Hg



An integral component of metabolic syndrome is blood pressure greater than 130/85 mm of Hg. Insulin resistance and hyperinsulinemia contribute to increased propensity for development of hypertension. Direct effect of elevated insulin on sympathetic nervous system activity can lead to elevated blood pressure.

In hypertensive patients, increased local formation of Angiotensin II in adipose tissues was noted [55] and therefore there exists close relationship between Angiotensin II and Insulin resistance.

The mean serum uric acid level was (6.53 mg/dl) in my study, with a maximum of (10.70 mg/dl) to a minimum of (3.41mg/dl). Many females, about 36 percent had serum uric acid levels between (6.00 – 6.90 mg/dl). GGT and uric acid showed positive correlation at (P=0.001) level.

The major component of metabolic syndrome [56] is insulin resistance, which influences protein metabolism, uric acid, an end product of protein metabolism is elevated. In Patient with metabolic syndrome , excretion of uric acid via kidney is also impaired .

In this study the mean, serum GGT level was (24.64) units/litre with maximum of (43.32) units/litre end a minimum of (5.90) units/litre. Among this, 46 percent showed high values of GGT ranging from (25.10 to 35.00) units/lit and 9 percent showed very high values (35.10 to 45.00) units /litre.

Eventhough GGT is expressed in several tissues, the main source of serum GGT is the Liver[57]. GGT's central role is in intrecellular glutathione homeostasis and extracellular glutathione metabolism. It enhances hydrolysis of gamma-

glutamyl bond of glutathione releasing dipeptide cysteinyl-glycine which outside the cell reduces Fe^{3+} to Fe^{2+} and releases a free thyolyl radical. This released free radical [58] oxidises LDL and promotes atherogenesis. In this way it acts as a Pro-oxidant in extracellular space.

Certainly elevations of serum GGT belong to the list of biomarker linked to metabolic syndrome. It appears to be largely a reflection of secondary hepatic inflammation. Although high level of GGT have been postulated to be directly atherogenic,[59] "Syndrome x" has strong associations with progressive Non-alcoholic fatty Liver Disease (NAFLD), age>45 years , obesity (BMI $\geq$ 30), Diabetes mellitus, $\frac{\text{AST}}{\text{ALT}}$ >1 etc., which increased the risk of developing significant fibrosis of liver.

The frequency of nonalcoholic fatty liver disease in the general population is given as 3-58%[60], whereby the great variability is due to socio-economic differences (average value 20-23 percent). The development of non-alcoholic fatty liver disease is more closely correlated with obesity than with alcohol abuse and simultaneously can be the cause for elevation of GGT levels in the serum.

The transaminase levels are normal (or) slightly increased. Non alcoholic steetohepatitis[61] is mostly associated with obesity and (or) type II diabetes. Thus nonalcoholic steato hepatitis is regarded as a hepatic manifestation of metabolic syndrome. With nonalcoholic fatty liver disease, there is a rise in GGT levels.

CONCLUSION :

In this study on serum GGT levels in metabolic syndrome about 56 percent showed elevation in Gamma-Glutamyl Transferease levels which may be due to Non Alcoholic Fatty Liver Disease [61] which is the hepatic manifestation of metabolic syndrome.

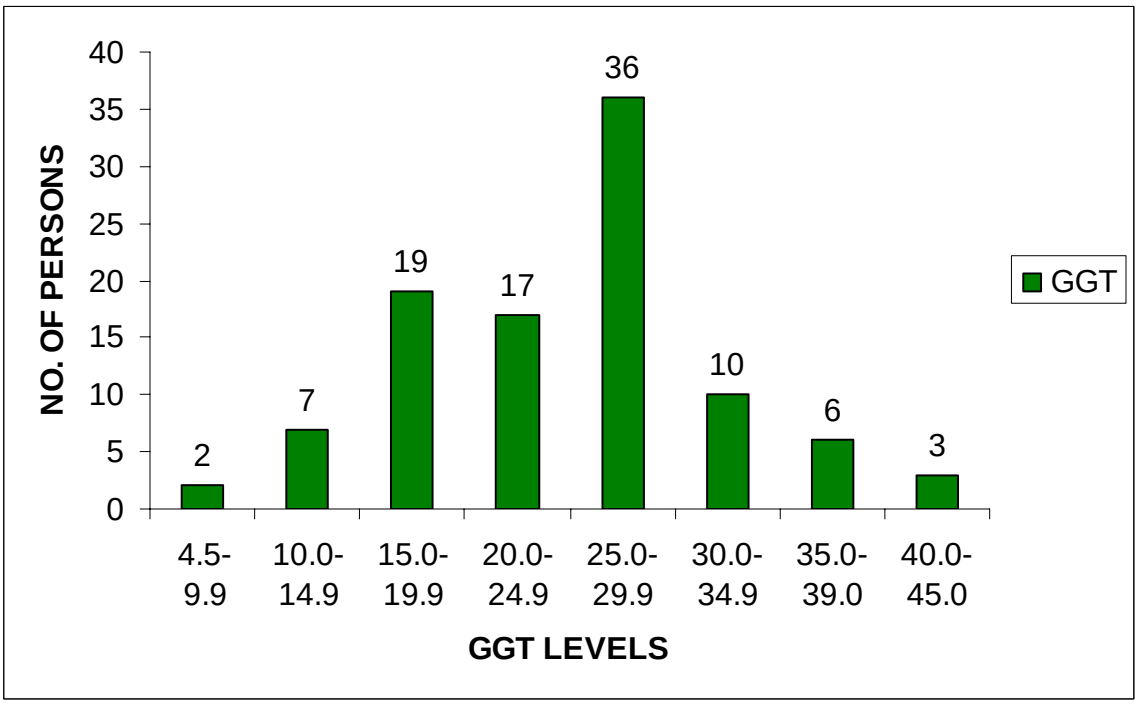
There is a very close relationship between low density lipoproteins, total cholesterol, triglyceride levels with serum GGT levels. Since LDL and GGT are independent risk factors for coronary heart disease, it will be very useful to describe GGT as a potential biomarker for coronary heart disease.

The earlier the patients with dyslipidemia [62,63] are to be investigated for elevated GGT levels and type-II diabetes mellitus. If the patients have elevated GGT levels with increased waist circumference, lifestyle modification can decrease the rate of progression to diabetes and coronary heart disease. Weight loss of 4kg over 3 years, 150 minutes of exercise per week, a low fatty and high fibre diet can be advised.

Dietary recommendations for people with metabolic syndrome can be advised - eat plenty of fruits and vegetables, avoid heavily processed foods rich in salt, sugar and fat.

Decreased salt intake and drugs for hypertension to be taken regularly to control blood pressure. ACE inhibitors can be recommended, for lowering the blood pressure as well as to improve the insulin sensitivity and decrease the rate of progression to diabetes mellitus.

Metabolic syndrome is a very wide topic with lot of prospects for future study and research into the various aspects of risk factors, interventions, and treatment modalities.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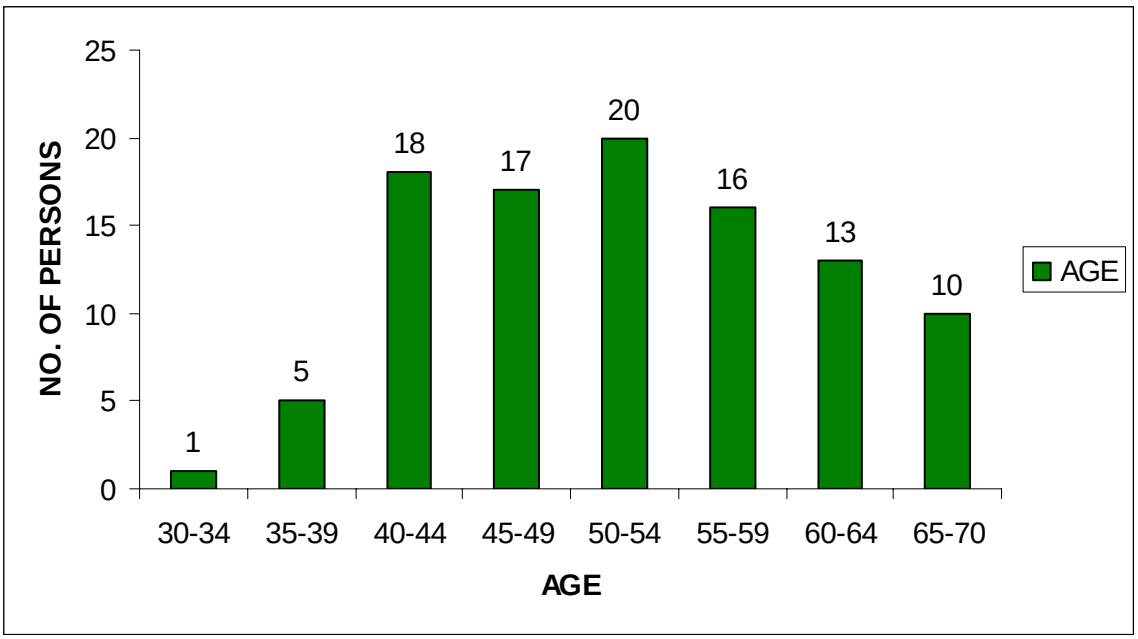
35
30
25
20
15
10
5
0
2
7
19
17
36
10
6
3
4.5-9.9
10.0-14.9
15.0-19.9
20.0-24.9
25.0-29.9
30.0-34.9
35.0-39.0
40.0-45.0
GGT LEVELS
GGT

Fig.(1)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10
5
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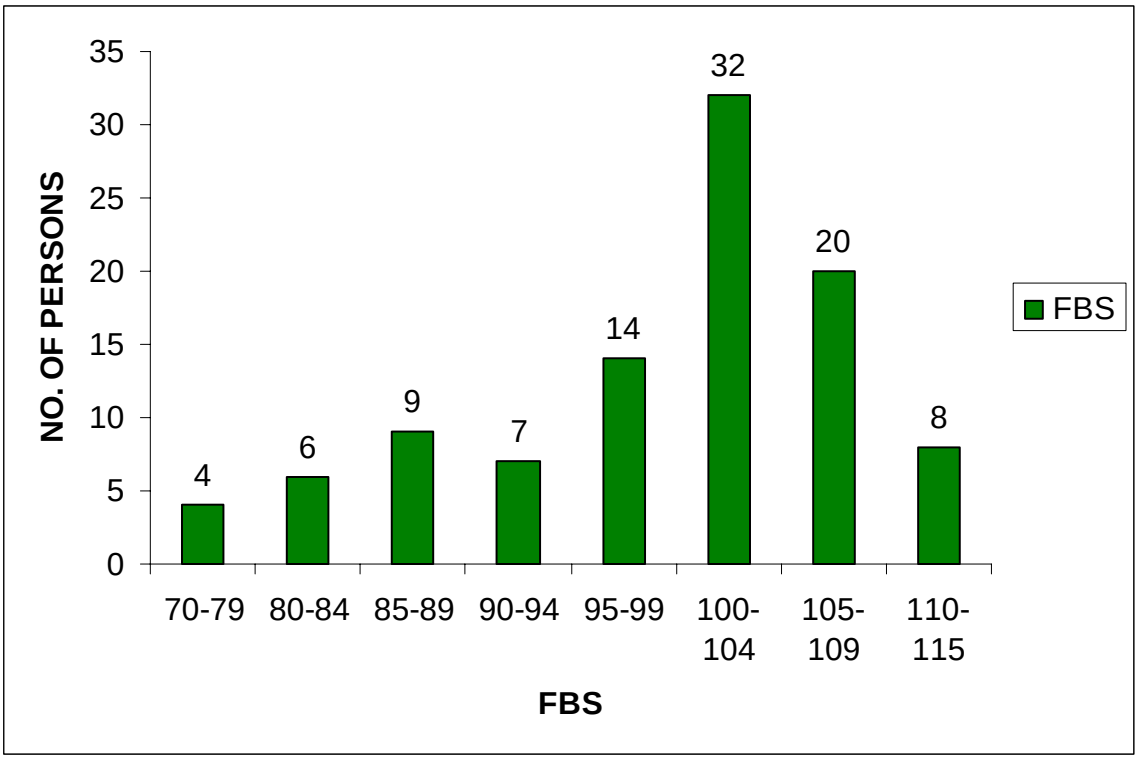
5
18
17
20
16
13
10
30-34
35-39
40-44
45-49
50-54
55-59
60-64
65-70
AGE
AGE

Fig.(2)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NO. OF PERSONS
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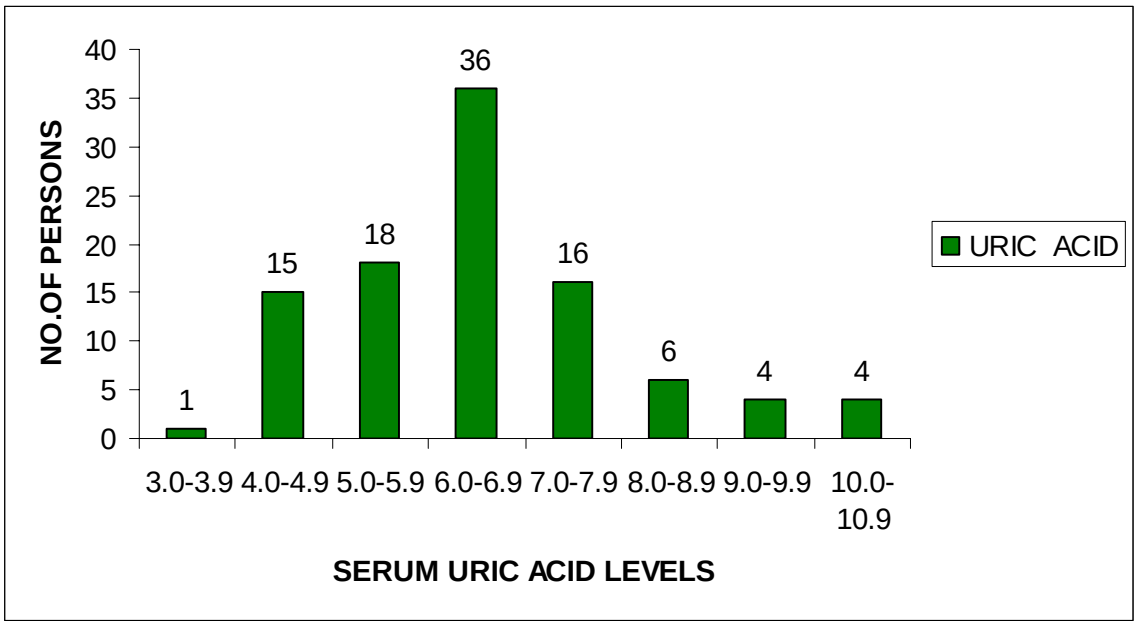
6
9
7
14
32
20
8
70-79
80-84
85-89
90-94
95-99
100-104
105-109
110-115
FBS
FBS

Fig.(3)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25
20
15
10
5
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NO.OF PERSONS
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15
18
36
16
6
4
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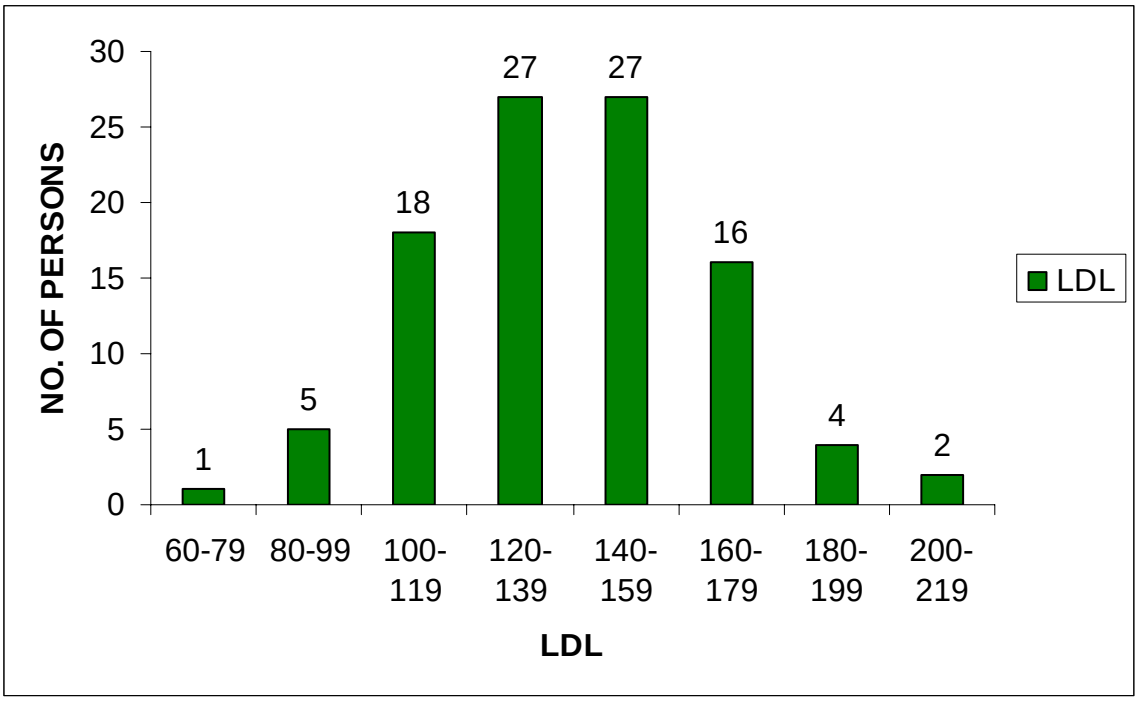
3.0-3.9
4.0-4.9
5.0-5.9
6.0-6.9
7.0-7.9
8.0-8.9
9.0-9.9
10.0-10.9
SERUM URIC ACID LEVELS
URIC ACID

Fig. (4)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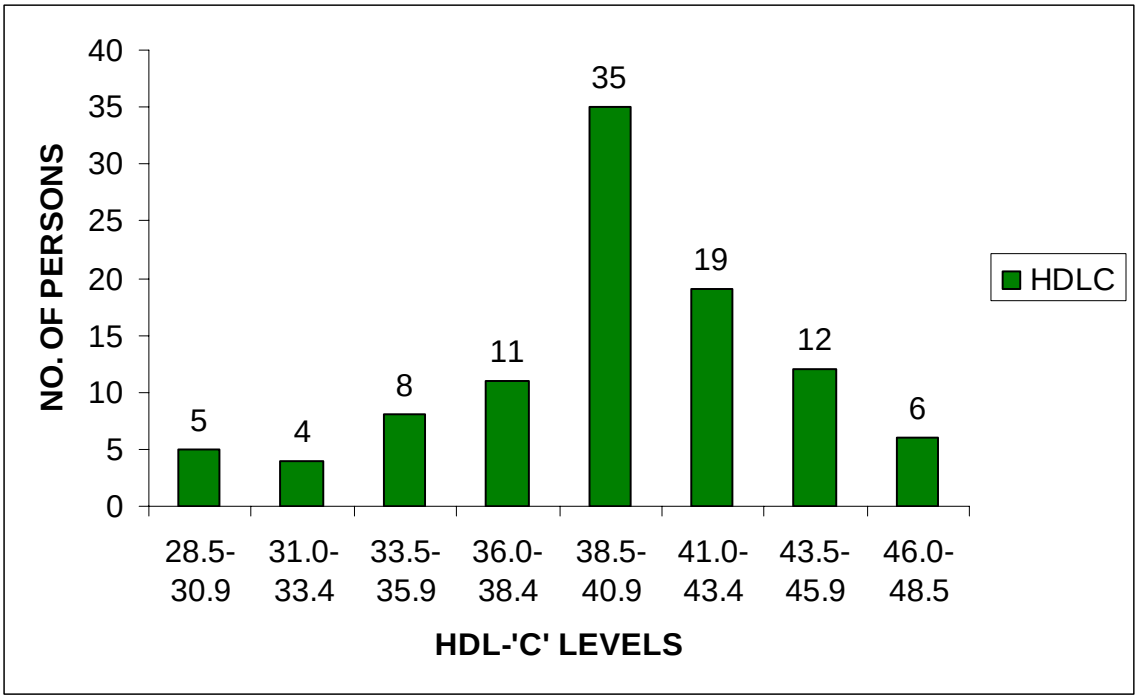
25
20
15
10
5
0
NO. OF PERSONS
1
5
18
27
27
16
4
2
60-79
80-99
100-119
120-139
140-159
160-179
180-199
200-219
LDL
LDL

Fig.(5)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25
20
15
10
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4
8
11
35
19
12
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31.0-33.4
33.5-35.9
36.0-38.4
38.5-40.9
41.0-43.4
43.5-45.9
46.0-48.5
HDL-'C' LEVELS
HDLC

Fig.(6)

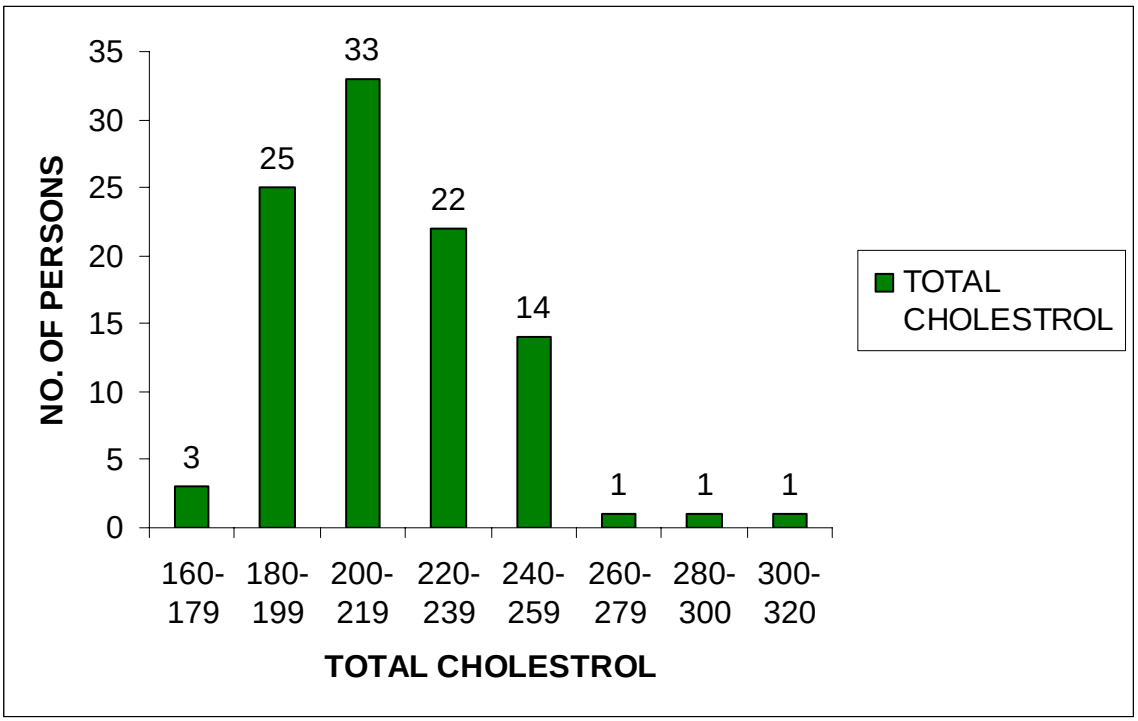
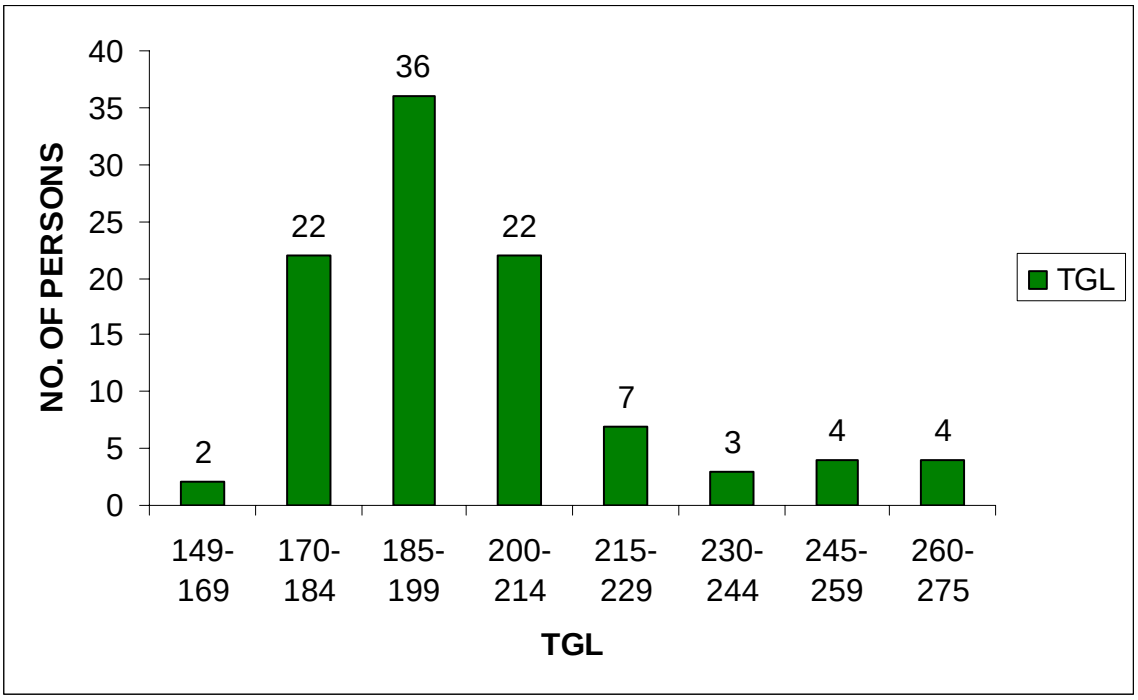

35
30
25
20
15
10
5
0
NO. OF PERSONS
33
25
22
14
3
1
1
1
160-179
180-199
200-219
220-239
240-259
260-279
280-300
300-320
TOTAL CHOLESTROL
TOTAL CHOLESTROL

Fig.(7)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35
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25
20
15
10
5
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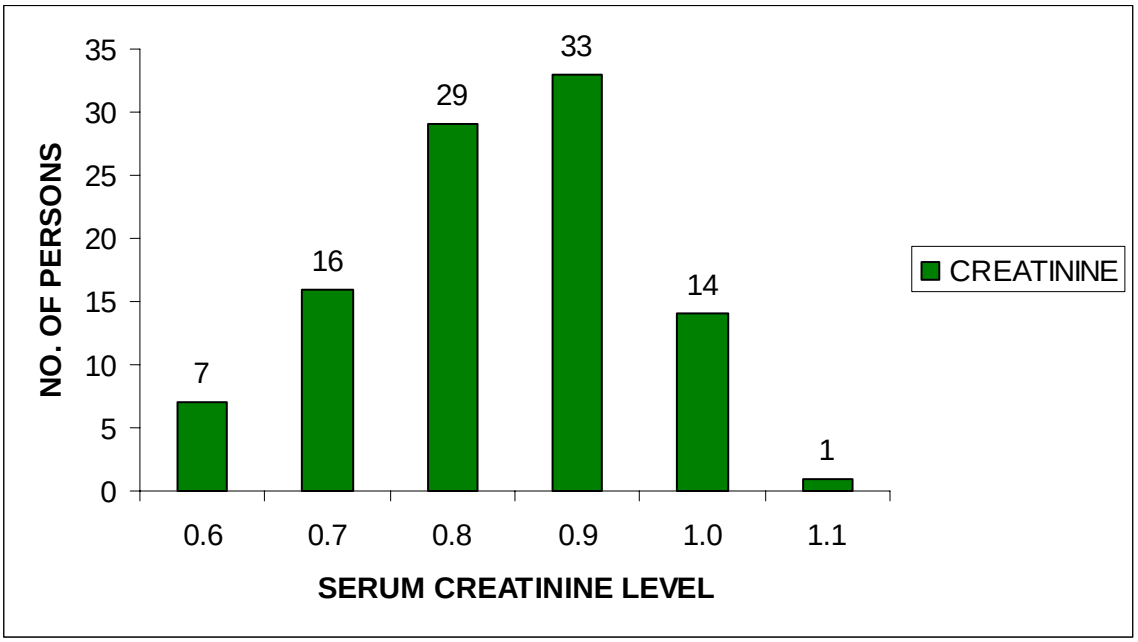
22
22
7
2
3
4
4
149-169
170-184
185-199
200-214
215-229
230-244
245-259
260-275
TGL
TGL

Fig.(8)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25
20
15
10
5
0
NO. OF PERSONS
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16
29
33
14
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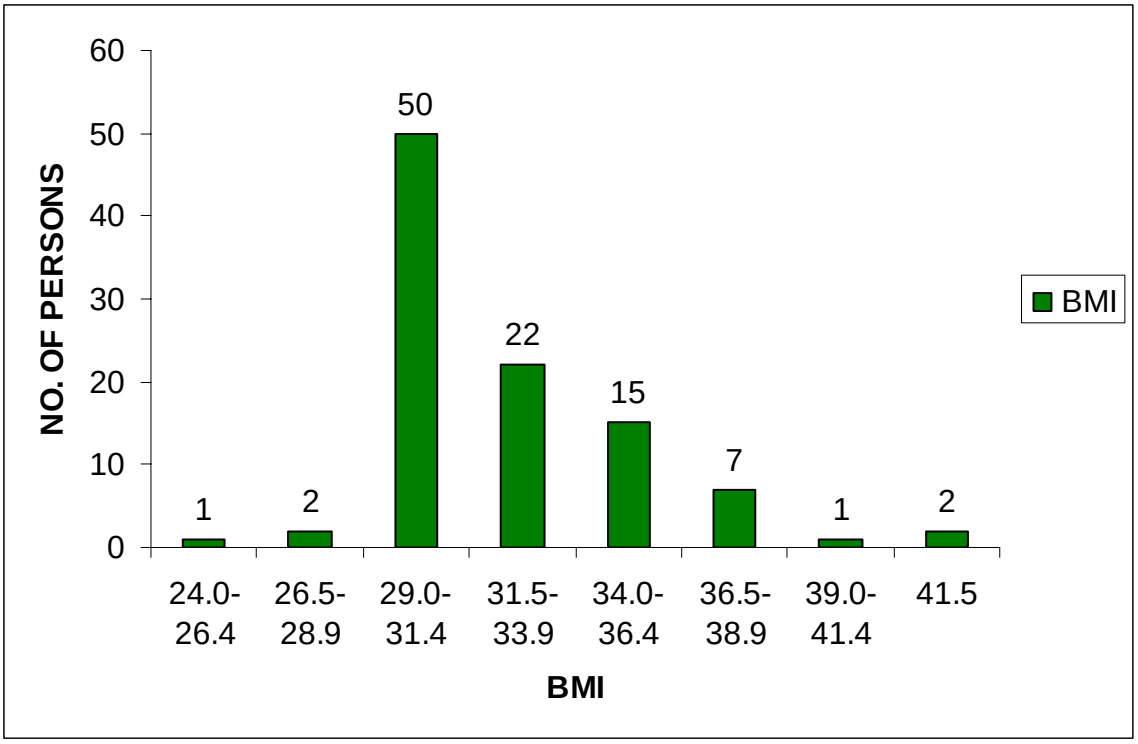
0.7
0.8
0.9
1.0
1.1
SERUM CREATININE LEVEL
CREATININE

Fig.(9)


60
50
40
30
20
10
0
NO. OF PERSONS
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50
22
15
7
1
2
24.0-26.4
26.5-28.9
29.0-31.4
31.5-33.9
34.0-36.4
36.5-38.9
39.0-41.4
41.5
BMI
BMI

Fig.(10)

RELIGION

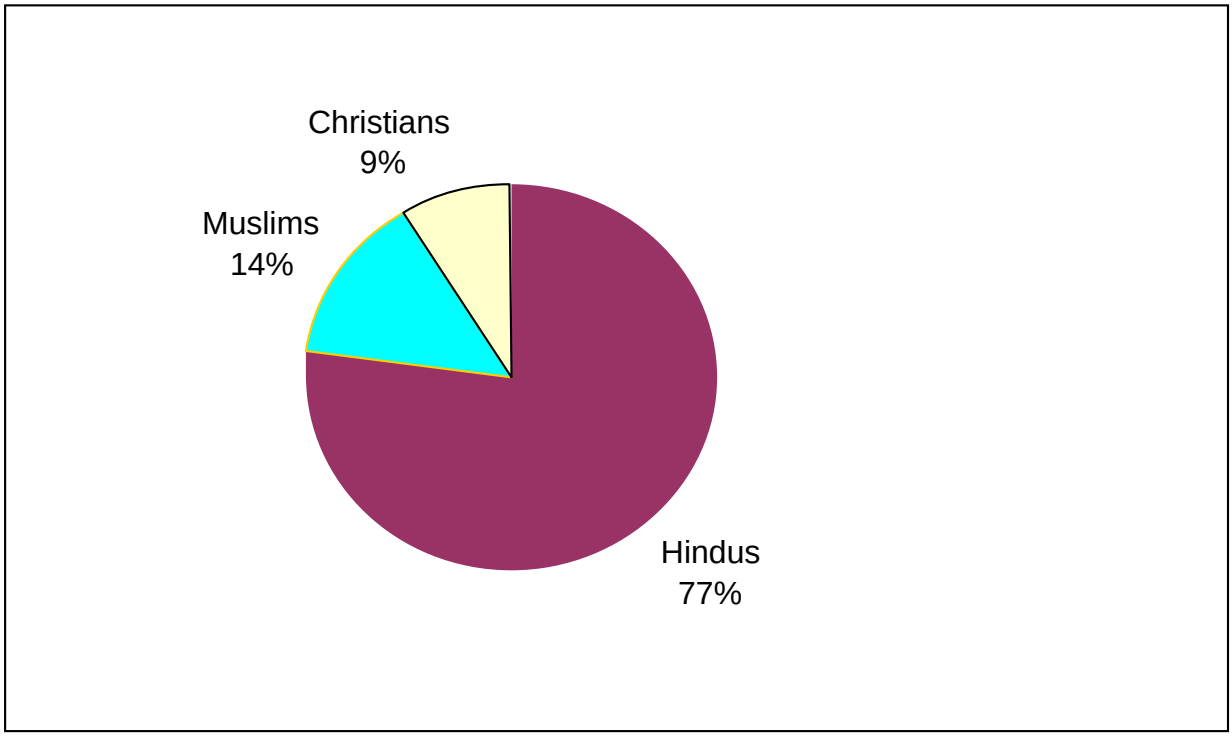

Fig. 11

GGT LEVELS

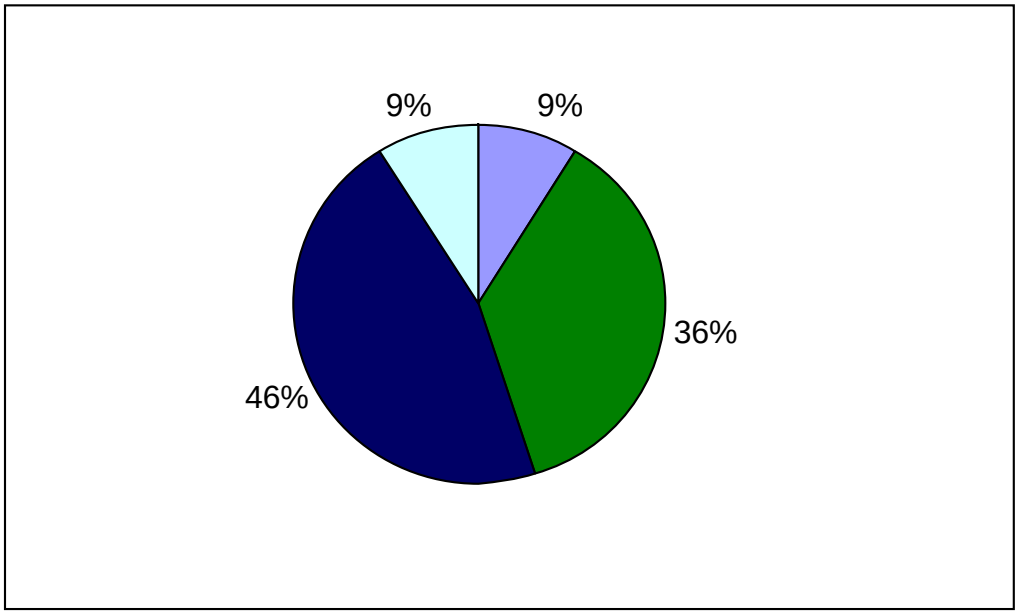

Fig. 12

LIST OF ABBREVATIONS

GGT	-	Gamma Glutamyl Transferrase
W.H.O.	-	World Health Organization
NCEP	-	National Cholesterol Education Programme.
BMI	-	Body Mass Index
FBS	-	Fasting Blood Sugar
HDL	-	High Density Lipoprotein
TGL	-	Triglycerides
TCL	-	Total Cholesterol
SGOT	-	Serum Glutamate Oxalo Acetate Transaminase
SGPT	-	Serum Glutamate Pyruvate Transaminase
AST	-	Aspartate Trans Aminase
ALT	-	Alanine Transaminase
TNF	-	Tumor Necrosis Factor
VD	-	Vascular Disease
MS	-	Metabolic Syndrome
VLDL	-	Very Low Density Lipoproteins
LDL	-	Low Density Lipoproteins
POD	-	Peroxidase
DHBS	-	Dichloro Hydroxy Benzene Sulfonic Acid
MDH	-	Malate Dehydrogenase
PNPP	-	Para Nitro Phenyl Phosphate
NAD	-	Nicotinamide Adenine Dinucleotide
NAFLD	-	Non Alcoholic Fatty Liver Disease

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