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BIOCHEMICAL MARKER ANALYSIS IN OBESE PATIENTS

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ABSTRACT

Obesity is a chronic medical disorder that can lead to diabetes, high blood pressure, heart disease and other chronic illnesses. It is difficult to treat and has a high relapse rate. The aim of our study is to assess the clinical and biochemical parameters related to obesity and Cardio Vascular Disorders risk factors in type 2 diabetics and its correlation with body weight. About 10 patients of different age groups were selected as experimental materials. The study was intended to study the important blood markers which can help to diagnose the severity of the obesity. The markers of interest were serum cholesterol, triglycerides and Vitamin B12 levels. Vitamin B12 is used as mode of treatment for the obese patients. All these are found to be positively correlated. Serum cholesterol is measured by spectrophotometry, Triglycerides by GPO/POD method and Vitamin B12 by chemiluminiscent immunoassay methods. The results obtained were found to be significantly correlative especially in the age group 31-35 followed by 16-20 age groups. The vitamin B12 levels were inversely proportional to the levels of the obese values. This is revealing that, Vitamin B12 is a limiting factor in obese patients and can be used as remedial measure. All the results were confirmed and significance was given by the student's t test ($p<0.05$).

INTRODUCTION

Coronary artery disease has many risk factors, some of them are modifiable and others are non-modifiable. C Reactive Protein (CRP) is considered as one of the non-specific inflammatory marker which is raised in inflammatory conditions, infections and in coronary artery disease (CAD). Obesity is another strong risk factor for cardiac problems. At increasing BMI is directly proportional to the cardiovascular risk increases by the deposition of visceral fat in mesenteric and omental regions. Visceral fat accelerates the process of

atherogenesis and hence the future risk of developing CAD and hence it is dangerous than subcutaneous fat. Triglycerides are one of the major components of plasma lipids and are commonly elevated among the type-2 diabetics, familial combined hyperlipidemia, and over-weight people contributing to cardiovascular risk due to atherogenesis.[1]

Many recent studies suggest that fasting triglycerides are considered to be independent risk factor for CAD but some researchers have stressed on non-fasting TG as strong predictor of CAD and death. The objective of this study was to determine the correlation of fasting triglycerides (TG), total cholesterol and vitamin B12 among pre-obese and obese patients without established diagnosis of coronary artery disease (CAD).

Obesity

Obesity can be defined by using the Body Mass Index (BMI). The BMI equals a person's weight in kilograms (kg) divided by their height in meters (m) squared. Since BMI describes body weight and height, thereby it is strongly correlated with total body fat content in adults.[2] An adult who has a BMI of 25 – 29.9 is considered overweight, and an adult who has a BMI over 30 is considered obese. To test the hypothesis that cholesterol levels greatly influences the obese state of the patients, we intended to study the biochemical parameters and blood markers for checking the obese levels in different age group patients.[3]

Cholesterol and Obesity

Cholesterol is present in almost all cells of our body for many physiological and metabolic functions. In high serum cholesterol (hypercholesterolemia), fats are deposited in blood vessels that make difficult for the blood to flow properly through the arteries and hence heart may not get the sufficient amount of oxygen, causing a heart attack or stroke.[4,5] Obesity is mainly due to intake of more calories of saturated fat or trans fat thereby increasing cholesterol levels in blood. In obesity -associated disease the increased amount of fat is found in the abdominal region. Obesity raises blood LDL (“bad” cholesterol) and lowers HDL (“good” cholesterol). Recent studies in Kinetic analysis of serum cholesterol after administration of radioactive cholesterol shows a significant correlation between Hypercholesterolemia patients and obesity.[6,7]

Triglycerides and obesity

Obesity has a direct link with the increased levels of triglycerides. Triglycerides are the primary fat storage form and also the main constituent for metabolic function in our system.[8]

People who are overweight tend to have much higher levels of these fats. Excess triglycerides are a condition called hypertriglyceridemia.[9]

In a study done in 2003, showed that obesity can increases intra-myocellular triglyceride peroxidation. Intra-myocellular triglycerides (IMTG) accumulate in the muscle of obese humans and are considered a pathogenic factor in the development of insulin resistance. In that study the lipid peroxidation/IMTG ratio was 4.2-fold higher in obese subjects than in subjects whose weight was within the normal range.[10]

Increased levels of triglycerides increases the risk of heart disease, diabetes, disorder related to pancreas, liver and kidneys. Researchers always mentioned in many studies that risk of having a heart attack or stroke is especially when there exist an increased level of triglycerides accompanied by obesity and high cholesterol. Triglycerides may also block a hormone that controls appetite and burning of calorie. To lower triglycerides levels, it is important to reduce body weight and also fatty and processed foods.

Researchers from the St. Louis University School of Medicine reported in the May 2004 issue of "Diabetes" that high triglycerides may block the work of the hormone leptin. Leptin helps to keep our body to shut down our appetite after you eat and to burn more calories when you have too much stored energy.[11] Triglycerides inhibit the hormonal function of leptin.[12]

Vitamin B12 and Obesity

Vitamin B12 is an essential nutrient to maintain our body metabolism especially in the synthesis of DNA during cell division and in the formation of healthy Red Blood Cells (RBCs) etc. The dietary sources are Meat, Poultry, Milk and other dairy products, Eggs, and Fish. It plays a significant role in decomposing the fat and protein to produce energy and hence it helps in weight loss indirectly. However, Vitamin B12 is not sufficient to treat obesity disorder solely. There Human body needs about three micrograms of vitamin B12 daily. The supplementary vitamin doses contain B12 in the percentage of hundreds. But, it is a water soluble component. So, the unused vitamin comes out of the body through urine. Researchers from the Bastyr University Research Institute conducted a 10-year study among adults, assessing how well several supplements, including vitamin B-12, worked in controlling their weight. The participants were in their 50s, an age group associated with

common B-12 deficiencies. Among overweight or obese men and women, long-term use of B-12, along with B-6 and chromium, was associated with lower levels of weight gain.[13]

MATERIALS AND METHODS

HDL levels

The LIASYS HDL Cholesterol Auto-Easy Reagent is an immune inhibition reagent method which directly measures serum HDL-Cholesterol levels. There are two reagents in this method. The Reagent A Antihuman lipoprotein antibody binds to lipoproteins (LDL, VLDL, & chylomicrons) other than HDL. The antigen antibody complexes formed block enzyme reactions when Reagent B is added. The Reagent B contains enzymes which then selectively react with the cholesterol present in the HDL particles. And hence the only HDL cholesterol is subjected for cholesterol measurement. Cholesterol esterase & cholesterol oxidase in Reagent B reacts with HDL-C. Upon oxidative condensation a blue colour complex is formed which is read at 593nm.

Equipment: Mindray BS380

Control

Randox Human assay Control 2(717UN) & 3 (472UE) (Ref-HN 1530 & HE 1532)

System Parameters: HDL (Immunoinhibition)

Temperature	37°C
Primary Wavelength	600 nm
Secondary Wavelength	700 nm
Assay Type	Endpoint
Direction	Increase
Sample Volume	4 µL
Reagent 1 Volume	300 µL
Incubation Time	5 mins
Reagent 2 Volume	100 µL
Incubation Time	5 mins

Sensitivity / Limit of Detection

A calibration factor of 514.390 was obtained, which is equivalent to a sensitivity of 0.002 ΔAbs. per mg/dL.

The lower Limit of Detection was found to be 3.0 mg/dL

Parameters calculated using the final results

LDL Cholesterol = Total cholesterol - (HDL Cholesterol + VLDL Fraction)

Total Cholesterol: HDL ratio = Total Cholesterol/HDL

LDL Cholesterol: HDL Cholesterol Ratio = LDL/HDL

Interpretation

Low risk : >60 mg/dl

Normal risk : 35 – 60mg/dl

High risk : < 35 mg/dl

Decreased HDL Cholesterol concentrations are positively correlated with the incidence of atherosclerotic diseases, basis of myocardial infarction and cerebrovascular accidents. There are several disease states or environmental influences associated with reduced levels of HDL such as acute or chronic hepatocelluar diseases, intra venous hyperalimentation, severe malnutrition, diabetes, chronic anemia, myeloprofilerative disorders, acute stress and smoking.

Serum cholesterol levels

Endogenous Cholesterol is mainly synthesized in the liver and gets transported in the blood in the form of LDL and HDL. In blood, cholesterol is present in two forms, free and esterified form. Cholesterol esterase hydrolyses serum cholesterol esters and the free cholesterol released is then oxidized by cholesterol oxidase releases ketone which liberates hydrogen peroxide. Enzyme peroxidise converts the liberated hydrogen peroxides into water and oxygen. Para amino phenazone (4 aminophenazone) takes up the oxygen and together with phenol forms a pink coloured quinoneimine dye, which can be read at 515nm (yellow green filter).

Interpretation

Serum Cholesterol: 150 – 250 mg/dl

Serum cholesterol is increased in hypothyroidism, diabetes mellitus, nephrotic syndrome etc. Elevated serum cholesterol is a serious risk factor for the development of coronary artery disease. Decreased serum cholesterol is seen in severe hepatoclellular disease, hyperthyroidism and anaemia.

Serum Triglycerides levels

Triglycerides are sequentially hydrolysed by lipases to form Di, Monoglycerides and glycerol. The glycerol that are formed by lipases are acted upon by Glycerol Kinase (GK) in the presence of ATP to form Glycerol-3-Phosphate, which are then oxidised by G-3-Phosphate to form Dihydroxy acetone phosphate and hydrogen peroxide. The hydrogen peroxides that are liberated are acted upon by Peroxidase (POD) to oxidise 4-Aminoantipyrine and TOOS (N-ethyl-N-Sulphohydroxy propyl-mToluidine) to form a purple coloured complex which are read at 546 nm.

Interpretation

Normal Values

Male : 65 – 190 mg/dL

Female: 45 – 170 mg/dL

Elevated levels are found in atherosclerosis, diabetes mellitus, glycogen storage diseases like Von Gierke's disease, secondary hyperlipoproteinaemia, nephrotic syndrome etc.

Vitamin B12

Normal level of human serum ranges a few hundred micro-microgrammes (,u,ug.) of vitamin B12 per ml and hence a specialized techniques are necessary to quantify. Almost all the known methods employ microbial models as certain micro-organisms require vitamin B12 for its growth in a defined media. The requirement of vitamin B12 in nutrient media varies according to the organism used and the concentration of vitamin B12 is proportional to the amount of growth that can be measured either by turbidimetrically or by changes in pH of the medium. Chemiluminescent immunoassay for the quantitative determination of vitamin B12 levels in human serum and plasma.

Equipment **:** Access 2

Reference Value : 180 – 914 pg/ml

Detection limit : 50 – 1500 pg/ml

For values higher than detection limits, sample is diluted with equal amount of distilled water and the measurement repeated. The obtained value is multiplied by 2 to arrive at the actual value. The calculations are recorded by the technologists in the work sheets.

Interpretation

Vitamin B12 functions as a coenzyme and involved in very important metabolic functions and DNA synthesis. Deficiency of vitamin B12 can lead to megaloblastic anemia, pernicious anemia, gastrectomy, a variety of bacterial or inflammatory diseases affecting the small intestine and also in neurological disorders.

RESULTS

Serum cholesterol levels

Table 1: Serum cholesterol levels from the patients (mg/dL). The values are the means of the experimental samples.

Age Group	Average (mg/dL)
16-20	204
21-25	143
26-30	137.5
31-35	270.5
36-40	133

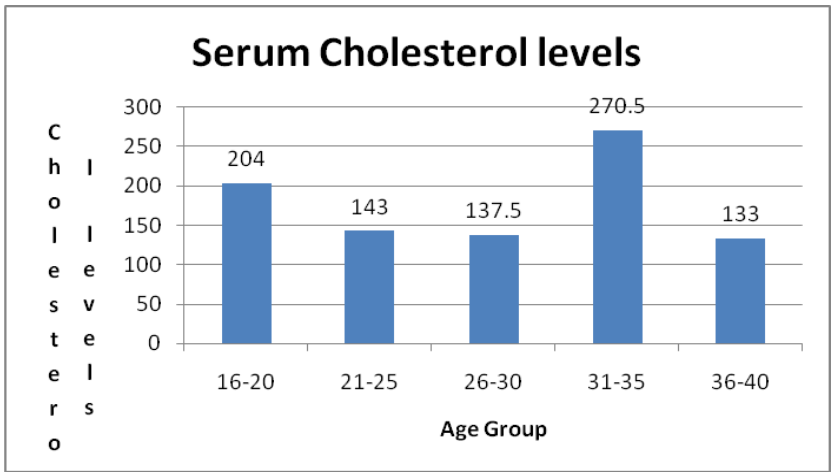

Fig 1: Graph showing the cholesterol levels in serum of different age groups.

Table 2: Statistical report of the cholesterol levels, done on Students t test, paired mean samples.

t-Test: Paired Two Sample for Means		
	Variable 1	Variable 2
Mean	3	177.6
Variance	2.5	3530.425
Observations	5	5
Pearson Correlation	-0.038586	
Hypothesized Mean Difference	0	
Df	4	
t Stat	-6.561708	

P(T<=t) one-tail	0.0013952	
t Critical one-tail	2.1318468	
P(T<=t) two-tail	0.0027904	
t Critical two-tail	2.7764451	

Serum Triglycerides levels

Table 3: Serum Triglyceride levels from the patients (mg/dL) The values are the means of the experimental samples.

Age (Group)	Average (mg/dL)
16-20	280
21-25	205.3333
26-30	137
31-35	409
36-40	149

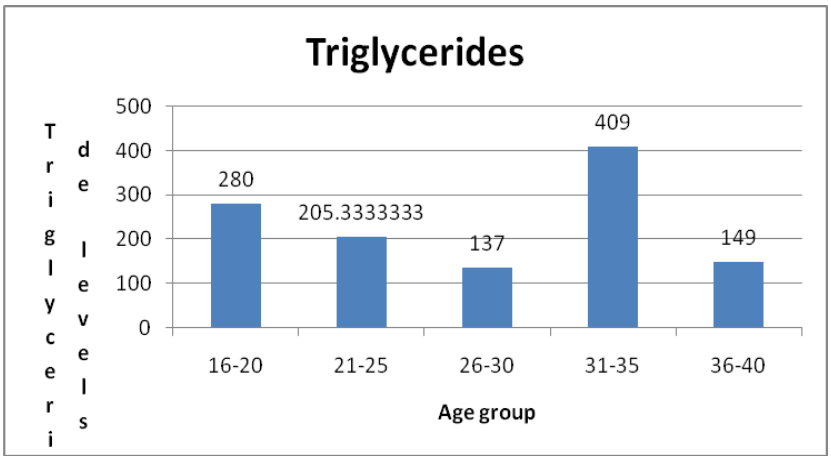

Fig 2: Graph showing the triglyceride levels in serum of different age groups.

Table 4: Statistical report of the Triglyceride levels, done on Students t test, paired mean samples

t-Test: Paired Two Sample for Means		
	Variable 1	Variable 2
Mean	3	236.06667
Variance	2.5	12543.856
Observations	5	5
Pearson Correlation	-0.082351	
Hypothesized Mean Difference	0	
Df	4	
t Stat	-4.647315	
P(T<=t) one-tail	0.0048404	
t Critical one-tail	2.1318468	
P(T<=t) two-tail	0.0096809	
t Critical two-tail	2.7764451	

Vitamin B12 Levels

Table 5: Vitamin B_{12} levels based on their age groups (pg/ml). The values are the means of the experimental samples.

Age Group	Average (pg/mL)
16-20	75
21-25	184.3
26-30	316.5
31-35	125.5
36-40	325

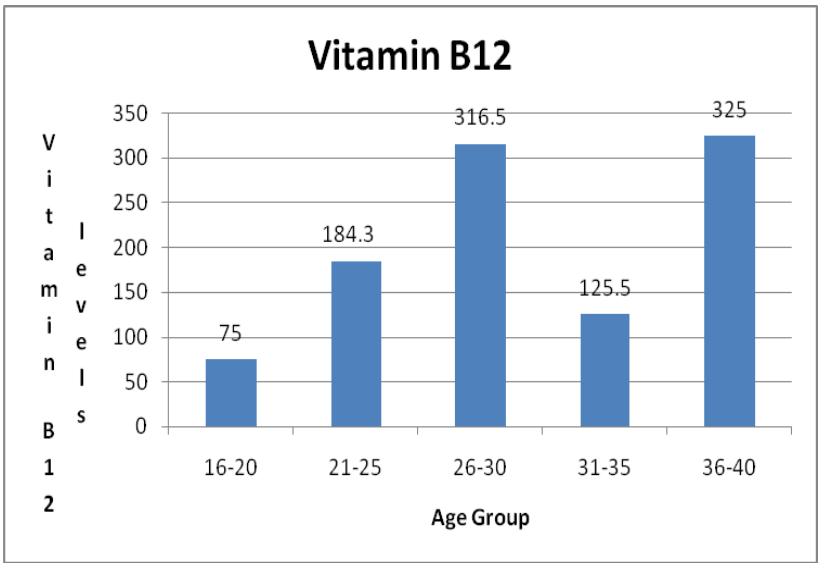

Fig 3: Graph showing the Vitamin B12 values of different age groups

Table 6: Statistical report of the Vitamin B12 levels, done on Students t test, paired mean samples.

t-Test: Paired Two Sample for Means		
	Variable 1	Variable 2
Mean	3	205.26667
Variance	2.5	12619.814
Observations	5	5
Pearson Correlation	0.6209351	
Hypothesized Mean Difference	0	
Df	4	
t Stat	-4.061329	
P(T<=t) one-tail	0.007666	
t Critical one-tail	2.1318468	
P(T<=t) two-tail	0.0153319	
t Critical two-tail	2.7764451	

DISCUSSION

Serum cholesterol levels

Cholesterol is an important constituent in building cells within the human body. The majority of cholesterol in the body is synthesised in the liver, and the rest are from the dietary sources that we ingest. Unhealthy cholesterol levels will affect blood vessels and cardiovascular system and is a significant risk factor for cardiovascular disease.[3]

Blood cholesterol profiles differed significantly in males and females. LDL: Cholesterol (2.66 mmol/L) and the TG: HDL Cholesterol ratio (3.3) were both lower in females compared to males (LDL Cholesterol: 2.93 mmol/L, TC: HDL Cholesterol ratio: 4.1). HDL Cholesterol was higher in females (1.53 mmol/L) compared to males (1.23 mmol/L).

It was determined that 40% of individuals aged 31 – 35 and 16 – 20 were living with unhealthy LDL Cholesterol levels and 26% of individuals were living with an unhealthy TC:HDL Cholesterol ratio (Table: 1 and Fig.1). The 31 – 35 age groups had a significantly higher percentage of individuals with an unhealthy TC: HDL –C ratio (20%) compared to any other group.

Serum Triglycerides levels

Serum triglyceride (TG) level is usually increased in individuals who are obese or have type 2 diabetes.[2] Although the causes of cardiovascular disease, obesity, metabolic syndrome, and type 2 diabetes are complicated, increased serum TG level is common to both obese and type 2 diabetic individuals**.** Epidemiologic studies shows that TG levels is an independent risk factor for cardiovascular disease and stroke.[14] Hypertriglyceridemia indicates the abnormalities of lipid metabolism and insulin function. In this study serum cholesterol levels were found higher in the age group of 31 – 35 followed by 16 – 20. The triglycerides level in the 16 – 20 age groups is attributed to the diet style of the age group which included the teens also. Junk food consumption is one of the main reasons behind this issue. 31 – 35 age group high level of triglycerides shows that the metabolic disorder for glyceride metabolism is higher and lack of exercise aggravating the issue.

The present results showed that there were 60% patients with normal triglyceride levels. This signifies that CRP may be raised in obese patients who cause a low grade inflammation in the arterial walls of obese patients and it can initiate a cascade of atherogenesis and hence poses a threat of coronary artery disease even in the absence of dyslipidaemia.

Vitamin B12 Levels

Obesity and vitamin B12 deficiency are common health problems encountered in primary care settings. Obesity is associated with many diseases. People with obesity are at great risk for certain diseases such as diabetes mellitus, cardiovascular diseases etc. In patients with obesity many inflammatory cytokines, such as interleukins, tumor necrosis factors, and C-reactive proteins, are increased [2]**.** A number of diseases that are not considered to be caused by vitamin B12 deficiency but epidemiologically associated with it, raises questions of whether vitamin B12 status may be considered as an independent risk factor or a partial causal agent. But all of these causal links are under active investigation.[14]

The total amount of about 2 – 5 mg vitamin B12 is stored in the body in adults. Approximately 50% of vitamin B12 is stored in the liver**.** Vitamin B12 deficiency especially among vegetarian people is usually due to pernicious anemias which are caused due to inadequate intake of nutrition enriched with vitamin B12. Various normal ranges for vitamin B12 have been propounded. Currently, there are no concrete levels for vitamin deficiency. A recent widely used clinical level for vitamin B12 deficiency was 148 pmol/L (200 pg/mL).[14]

Vitamin B12 deficiency results from an inadequate intake of nutrition, abnormal nutrient absorption, and rare inborn errors of vitamin B12 metabolism. Vitamin B12 is mainly present in animal protein, particularly organ meats, bivalves, and, to a lesser extent, in seafood, milk, and milk products. However, prevalence of vitamin B12 deficiency is not rare, and it is especially common the elderly. Vitamin B12 deficiency does not only cause anemia and megaloblastic anemia, but also causes neurological symptoms. Recent studies have shown that longterm use of metformin decreases vitamin B12 levels in patients, especially diabetic patients**.** Therefore, we excluded the patients who had used metformin for a long period of time.

A correlation between obesity and vitamin B12 levels has been investigated in a few studies. In our study Age group of 36 – 4 0 is found to be significant lower B12 followed by 26 – 30. Han, T. S et al conducted a study on plasma homocysteine and lipoprotein levels in patients and found low levels of vitamin B12 compared to normal healthy individuals.[15] We studied vitamin B12 and folic acid levels, but not homocysteine levels, and we also found that vitamin B12 levels were low but that folic acid levels were normal.

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