Chapter three

Materials and Methods

3.Materials and Methods

3.1 Study design

This was a descriptive analytical cross-sectional case control study.

3.2 Study population

This study included 90 Sudanese subjects classified as 30 obese,30 overweight and 30normal weight as control group their age range from (18-40) years old .

3.3 Study Area and period

This study was carried out in body master center in Khartoum state during period of September and October 2017.

3.4 Inclusion criteria

Non diabetic non hypertensive Sudanese individual with BMI greater than 25 were enrolled in this study.

3.5 Exclusion criteria

Subject with Acute infectious diseases including periodontal and urinary infections, and malignancy, Alcohol consumption, chronic disorders of the Joints and connective tissues, Pregnancy, renal diseases,Cigarette smoking and liver diseases were excluded in this study.

3.5 Ethical consideration

Study was approved from local ethical committee of the Sudan University of Science and Technology, verbal informed consent was obtained from all participants after informed by the aims of the study.

3.6 Collection of Samples

Samples were collected using dry plastic syringes, tourniquet was used to make the veins more prominent, 5ml blood samples was collected in plane containers each volunteer was collected underseptic condition. All blood samples in plane containers were allowed to clot at 25°, and then they were centrifuged at 4000 rpm to obtain the serum samples, and stored in -20° until the analysis.

3.7 Estimation of serum total cholesterol :

3.7.1 Principle of method:

Ester cholesterol hydrolyzed in present of cholesterol esterase to free fatty acid and free cholesterol which oxidized by atmospheric oxygen in presence of cholesterol oxidize to cholestene-3,1 and hydrogen peroxide , which converted by peroxidase to H 2O and oxygen then oxygen accepted by para amino phenazone in presence of phenol to produce quinoninmine pink color measured by spectrophotometry. (Allain*et al.*, 1974).

3.7.2 Procedure: Appendix VI

3.8 Estimation of Triglycerides :

3.8.1 Principle of method :

Triglycerides hydrolyzed enzymatically in the presence of lipase to 3fatty acid and glycerol , which phosphorylated in the presence of ATP and glycerol kinase to glycerol-3-phosphate that oxidized in presence of glycerol-3-phospate oxidase to dihydroxyacetone phosphate and hydrogen peroxidewhich converted by peroxidase to H 2O and oxygen then oxygen accepted by para-amino phenazone in presence of phenol to produce quinoninmine pink color measured by spectrophotometry. (Fossati and Prencipe., 1982).

3.8.2 Procedure : Appendix VI

3.9 Estimation of high density lipoprotein (HDL-c):

3.9.1 Principle of method:

Very low density lipoproteins, chylomicrons and low density lipoproteins in the sample precipitate with phosphotungstate and magnesium ions, after centrifugation the supernatant contains high density lipoproteins which measured by cholesterol oxidase method spectrophotometrically. (Burstein *etal.,* 1980).

3.9.2 Procedure: Appendix VI

3.10 Calculation of low density lipoprotein (LDL-c):

LDL-c calculated from Fried-Wald's equation:

LDL-c = Total cholesterol – HDL-c –Triglyceride\5.(Bishop *etal*.,2004)

3. 11 Serum Magnesium

3.11.1 Principle

Magnesium form a purple coloured complex when reacts with calmagite in alkaline solution The intensity of the color formed is proportional to the magnesium concentration in the sample(Farrell,1984)

3.11.2 Procedure : Appendix V

3.12 CRP Estimation

3.12.1 Principle :

Particle enhanced immune turbidimetric assay .Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically (kindmarh,1972)

3.12.2 Procedure : Appendix VI

3.13 BMI calculation :

BMI obtained by calculation according to formula:
weight(kg) ÷ height (m^2) (Who ,2000)

3.14 Waist Hips Ratio :\Waist (cm)/hips(cm) (Geissler & Powers, 2005**)**

3.15 Quality control :

To ensure adequate quality control, The validity of the reaction monitored by use of control sera with known normal and abnormal controls level each run .

3.16 Statistical Analysis:

The data was analyzed using statistical package of social science (SPSS) computer program using frequencies, independent sample t test and Pearson correlation , results was expressed as percentage (%) and (mean ± SD), and significance difference was. consider as (P-value <0.05).