CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Prevalence of Inflammation Associated Diseases in Obese and Non-obese Subjects of Kahuta Region

by

Saeed Iqbal

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

Faculty of Health and Life Sciences
Department of Biosciences

2018

Dedicated to Almighty ALLAH and the Holy Prophet Muhammad (P.B.U.H)and
My Loving Family

CAPITAL UNIVERSITY OF SCIENCE & TECHNOLOGY
ISLAMABAD

CERTIFICATE OF APPROVAL

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Abstract

In last two decades, studies revealed not only the complexity of inflammatory process but also increased understating of it complex intracellular signaling control mechanism. Inflammation is the immune response of the human body against the threat stimulus, which could also import harmful effects resulting in damages to the human body. Inflammation can be triggered in response to stimulus including pathogenic infections, chemical substances, and an autoimmune disorder. It is not only the outcome of infectious diseases but strongly linked to all non-infectious diseases. Chronic inflammation are long lasting inflammation. It is considered one of the major causes of obesity. Obesity which is a multi-factorial disorder can cause diabetes, cardiovascular diseases, hypertension, psychiatric disorders, and gastrointestinal disorders or vice versa. All these diseases are also reported to be an outcome of inflammation. This study was planned to determine prevalence and correlation among inflammatory disorders in obese and non-obese individuals of kahuta region to determine how the obesity could trigger the onset of inflammation disease. Survey was conducted to acquire data and blood sample for biochemical testing. Prevalence and the correlation was determined using SPSS statistical analysis. Out of total of 381 persons were enrolled 50% were females and 50% were males. According to body mass index 6% were underweight, 40% were normal, 28% were overweight, 15% were obese class 1, 8% were obese class 2 and 3% were obese class 3. In overall population (14%) had diabetes, (65%) had cardiovascular disease , (34%) had gastrointestinal disorders, and (39%) had depression. SPSS statistical analysis shown positive correlation as well as negative correlation among inflammatory disorders and obesity. It is observed that inflammation and obesity has significant positive correlation, diabetes with obesity and inflammation were found negatively correlated, correlation of cardiovascular disease with inflammation and obesity was significantly positive where as correlation of cardiovascular disease with diabetes was found negative.

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4.1 WHO BMI Classification for Normal, Overweight& Obese Individuals. 23

Abbreviations

IL	Interleukins
TNF-α	Tumor Necrosis Factor Alpha
CVD	Cardiovascular Diseases
GI	Gastrointestinal
CRP	C - Reactive Protein
SPSS	Statistical Package of Social Science
BSR	Blood Sugar
TC	Total Cholesterol
BMI	BBody Mass Index
Bp	Blood Pressure

Chapter 1

Introduction

1.1 Background

Increased adipose tissue mass can define obesity[1]. Obesity is result of energy imbalance between calories intake and expenditure. Genetics, environment, socioeconomic status and individual decisions are the factors play a significant role in developing obesity. Risk factors include gender, age, urbanization, and high living standards, unhealthy dietary habits, dynamical lifestyle are the most contributive risks for the raised in prevalence of obesity in all over Pakistan[2] [3]. Many studies suggested that chronic low grade inflammation interplay between obesity and its comorbidities including atherosclerosis, Hypertension, Irritable bowel syndrome, Diabetes, Anxiety and depression shown in Figure 1.1 [4].

In last two decades, studies revealed not only the complexity of inflammatory process but also increased understating of it complex intracellular signaling control mechanism [5]. Inflammation is the immune response of the human body against the stimulus, which could have some harmful effects resulting in damages to the human body. Inflammation can be triggered in response to stimulus including pathogenic infections, chemical substances, and an autoimmune disorder. Inflammation is not only the outcome of infectious diseases but strongly linked to all non-infectious diseases [6]. Generally the inflammations are of two types, acute

inflammation induced due to tissue damaged by trauma, microbial invasion or toxic compounds. It starts rapidly and become severe in short period of time and symptoms may last for few days whereas the chronic inflammation is the slow, long lasting for prolonged period of several months to years.

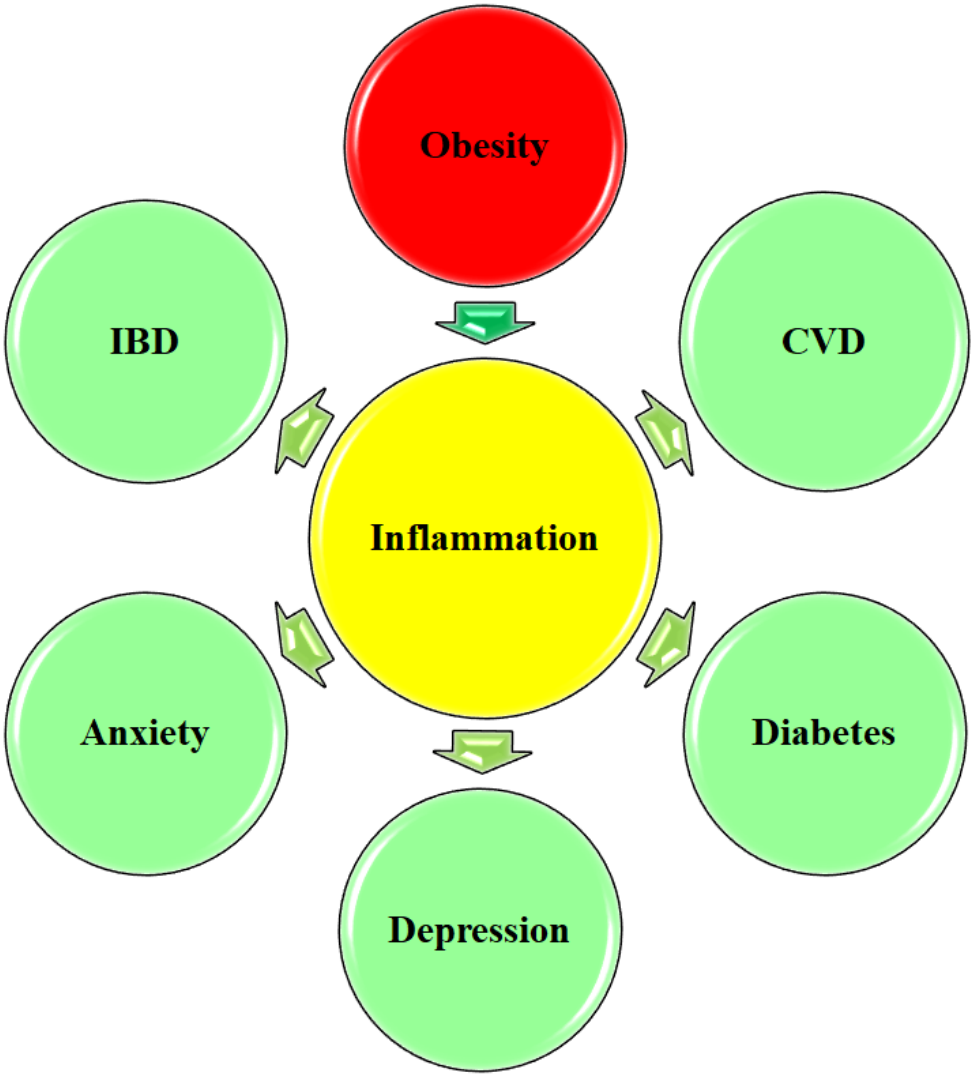

FIGURE 1.1: Association of Inflammation with obesity Cardiovascular Disease (CVD), Irritable bowel Syndrom (IBD), Diabetes, Depression and Anxiety .

Inflammatory response is the coordinated activation of signaling pathways to regulate pro and anti-inflammatory mediators in various cells [3]. Inflammatory process involves major immune system cells with neutrophils, basophils, mast cells, T-cells, B-cell, and leukocytes, etc. Inflammatory events are regulated by many extracellular mediators and regulators such as cytokines, growth factors, prostaglandins, leukocytes, complement, and peptides. Chronic inflammation is

considered one of the major causes of obesity. Complement activation and production of pro-inflammatory cytokines are the shared properties of immune cells and adipocytes[4], [5]. In addition, fat cell precursors and macrophages also have common feature such as numerous transcription factor coding genes, fatty acid transporters, inflammatory signaling molecules and capacity of phagocytosis [6]–[10]. Dynamic alteration in nutrient excess through adipocyte hyperplasia and hypertrophy, rapid responding endothelium, immune cells, stromal pre-adipocytes and of adipocyte's hetrogenous mix characterize adipose tissue [11]. It has been suggested that progressive adipocyte enlargement results in visceral obesity, causing a state of hypoxia has been considered the main trigger of micropgahes and necrosis inflitating in adipose tissue leading to the overproduction of adipocytokines [12], [13].

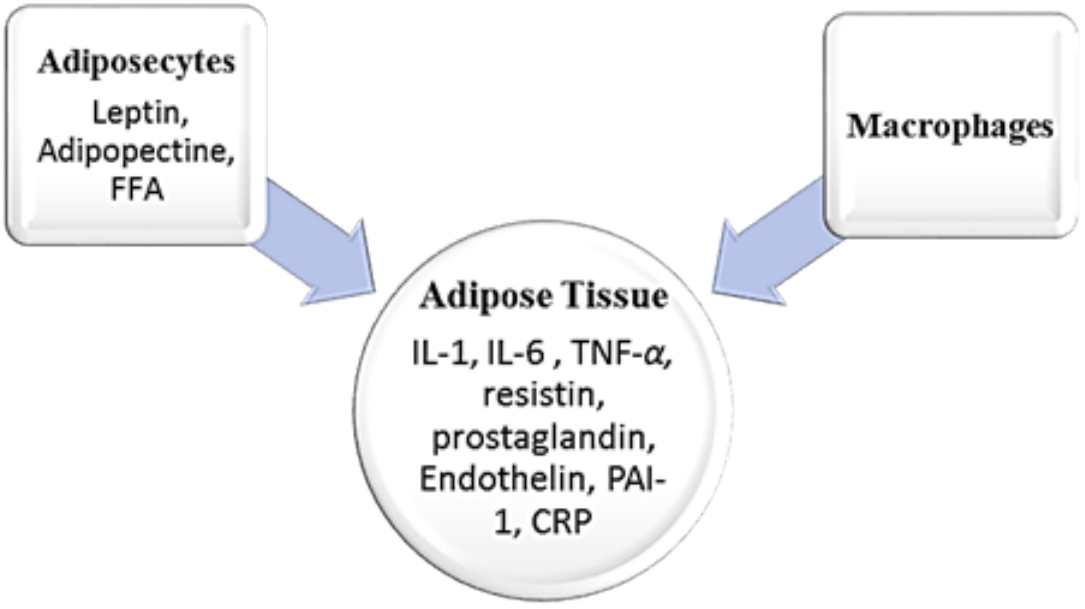

FIGURE 1.2: Secretion of Cytokines by Adipocytes and Macrophages.

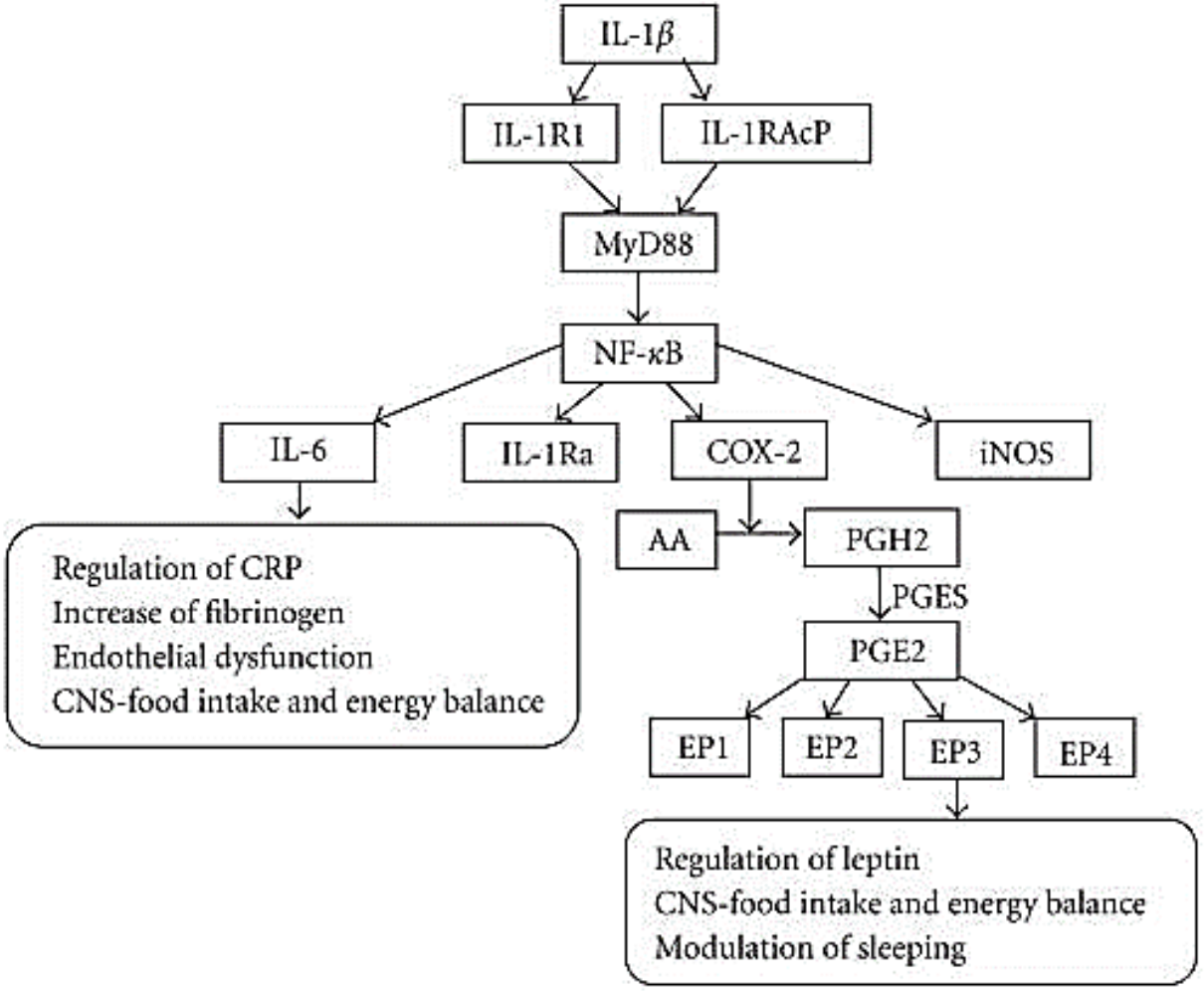

FIGURE 1.3: Inflammation Signaling Pathway [14].

1.2 Aims

Inflammation is considered one of the major outcomes of obesity, which in turn causes various inflammatory responses resulting in onset of co-morbidities such as cardiovascular diseases, irritable bowel syndrome, diabetes, hypertension and anxiety. This study is designed to estimate the prevalence of inflammatory responses and related diseases in obese subjects of Kahuta region.

1.3 Objectives

The study is designed with following major objectives:

- Prevalence of obesity in Kahuta region.
- Prevalence of inflammatory responses in obese individuals in comparison with non-obese individuals.
- Prevalence of inflammatory disorders in obese and non-obese subjects.

Chapter 2

Literature Review

2.1 Obesity

Increased adipose tissue mass can be defined as obesity[1]. Fat or triacylglycerol is the only factor that increases body weight as other energy storages like carbohydrates glycogen or proteins are readily utilized [19][20]. Obesity is highly associated with cardiovascular problem which leads to mortality [21]. Obesity has become worldwide pandemic [22].

TABLE 2.1: WHO BMI classification for Normal, Overweight& Obese Individuals.

BMI	Condition
>18-24.9 Kg/m^2	Normal Weight
>25-29.9 Kg/m^2	Overweight
>30 Kg/m^2	Obese
>30-34.9 Kg/m^2	Obesity Class I
>35-39.9 Kg/m^2	Obesity Class II
>40 Kg/m^2 or >35 Kg/m^2	Obesity Class III

Classification of BMI categories include in table 2.1 according to WHO Standards.

Obesity is result of energy imbalance between calories intake and expenditure. Genetics, environment, socioeconomic status and individual decisions are the factors play a significant role in developing obesity. Risk factors are included in figure 2.1.

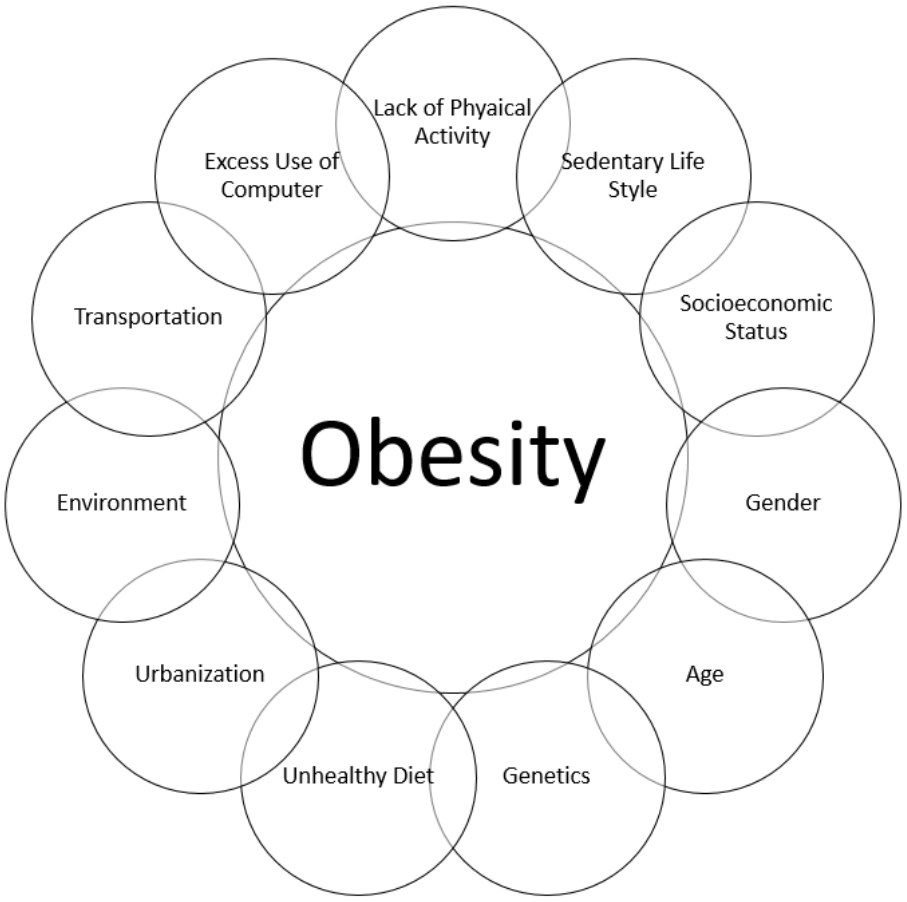

FIGURE 2.1: Risks Factors of Obesity [23][24].

2.2 Inflammation

Generally there are two types on inflammation acute inflammation induced due to tissue damaged by trauma, microbial invasion or toxic compounds. It starts rapidly and become severe in short period of time and symptoms may last for few days whereas the chronic inflammation is the slow, long lasting for prolonged period of several months to years. Acute inflammation play its role in healing significant particular site but the non-resolving chronic states of inflammation may

last for whole life of individual and causes asthma, bronchitis, atherosclerosis, inflammatory bowel syndrome, arthritis, osteoarthritis, diabetes, cardiovascular diseases, and psychiatric disorders[25][26][18]. Inflammation is considered one of the major outcomes of obesity, which in turn causes various inflammatory responses resulting in onset of co-morbidities such as cardiovascular diseases, irritable bowel syndrome, diabetes, hypertension and anxiety.

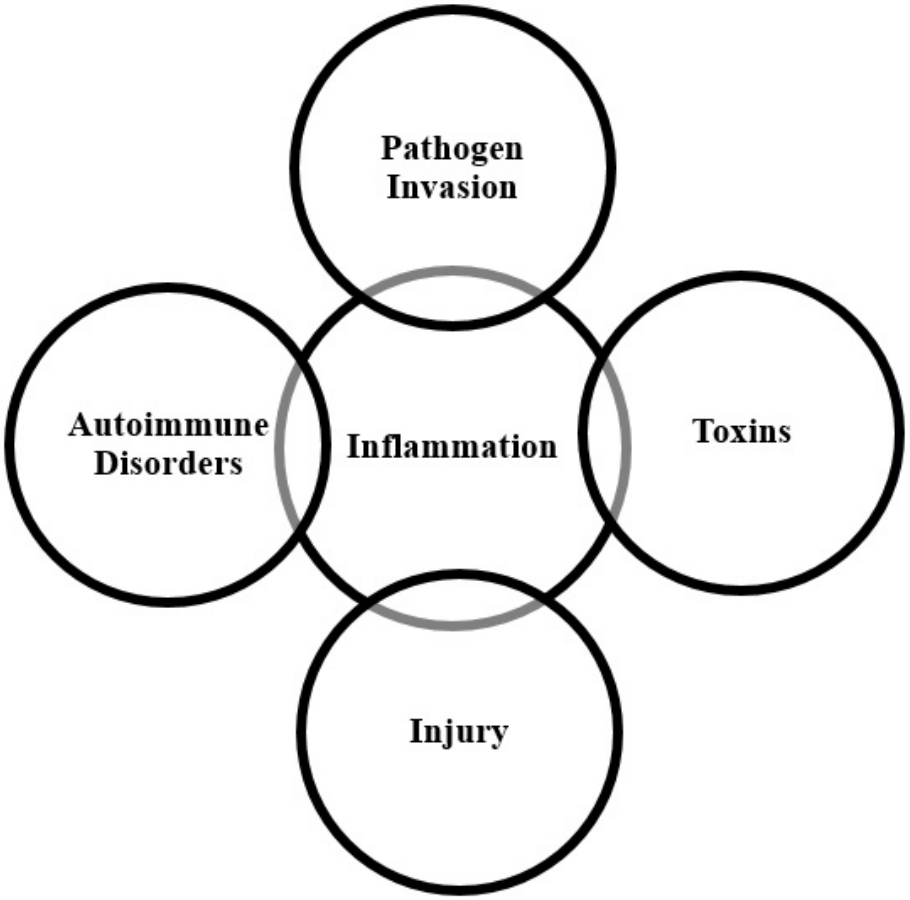

FIGURE 2.2: Inflammation Stimulus.

2.3 Cardiovascular Diseases

Cardiovascular diseases are the most critical health threat all over the world. CVD includes atherosclerosis vascular and heart diseases. Chronic inflammation is the link between the obesity and CVDs, Obesity induces inflammation in the arterial

TABLE 2.2: Blood Pressure Ranges for Hypertension

Blood Pressure	Systolic	Diastolic
Normal	<120	<80
Elevated	120-129	<80
High BP (Hypertension Stage 1)	130-139	80-90
High BP (Hypertension Stage 2)	140	90
High BP (Hypertension Stage 3)	180	120

wall of blood vessels. in obesity-mediated CVD adipose tissue is the key role player [27]. Adipocytes procure hormones peptides and some other molecules (inflammation mediators) to regulate inflammation pathways. Cholesterol-rich atherogenic Apo B lipoprotein transduces in the inner layer of blood arteries and leads macrophages and T-cells to interact with each other which ultimately induces inflammation in blood vessels [14].

Studies in experimental animals have provided mechanistic insights into CVD, as well as renal changes associated with obesity. Specifically, reproducible increases in systemic blood pressure have been identified in both dogs and rabbits fed fat diets that result in excess weight gain [30]–[33]. In fact, the metabolic, endocrine, cardiovascular, and renal changes caused by dietary-induced obesity in these experimental animals have closely mimicked changes observed in obese humans.

In addition to the potential mechanical compression caused by obesity that may mediate the development of hypertension, several other mediators of sympathetic nervous system activation have been proposed.

Establishment of hypertension as a primary component of Metabolic syndrome not only has allowed for earlier detection and proper management [28] but has also allowed for better understanding of the multifactorial etiology of this condition.

The metabolically active visceral fat linked to insulin sensitivity through the production of adipocytokines including leptin, tumor necrosis factor-a (TNF-a), angiotensinogen, interleukin-6 (Il-6), and non-esterified fatty acids (NEFA), interact

in a diversity of metabolic pathways culminating in the activation of the renin-angiotensin-aldosterone system (RAAS) pathway and the development of insulin resistance [29].

Surely adipose tissue is known to widely express angiotensinogen, angiotensin converting enzyme (ACE), and type 1 angiotensin receptor (AT1) gene, with the potential of increasing the overall production of Ang II and thus activate RAAS. While RAAS plays a key role in the modulation of many key cardiovascular functions, it is known that patients with the Metabolic syndrome have an altered up-regulation of RAAS resulting in chronic activation of inflammatory responses.

Furthermore, for over two decades, different subsets of Th1 interferon producing and Th2 interleukin-4 producing lymphocytes, as well as Th17 producing interleukin-17 and T-suppressor lymphocytes that participate as pro- and anti-inflammatory cells have been shown to participate in the process of vascular remodeling that occurs with hypertension. In addition, the role of pro-inflammatory T-lymphocytes has also been shown to mediate the effects of Ang II and mineralocorticoids in both Dahl-salt sensitive and spontaneously hypertensive rats.

Though the specific mechanism mediating this activation of immunity remains largely unknown, it has been proposed that formation of neo-antigens could be generated by elevated blood pressure through damage-associated molecular pathways. Moreover, Th1 cells once activated may contribute to increases in systemic blood pressure through the interaction of cytokines produced or through their effects on perivascular fat.

Obviously, confirmation of these mechanisms in humans might provide new therapeutic venues not only to change our current approach to managing hypertension but also how we can improve CVD outcomes.

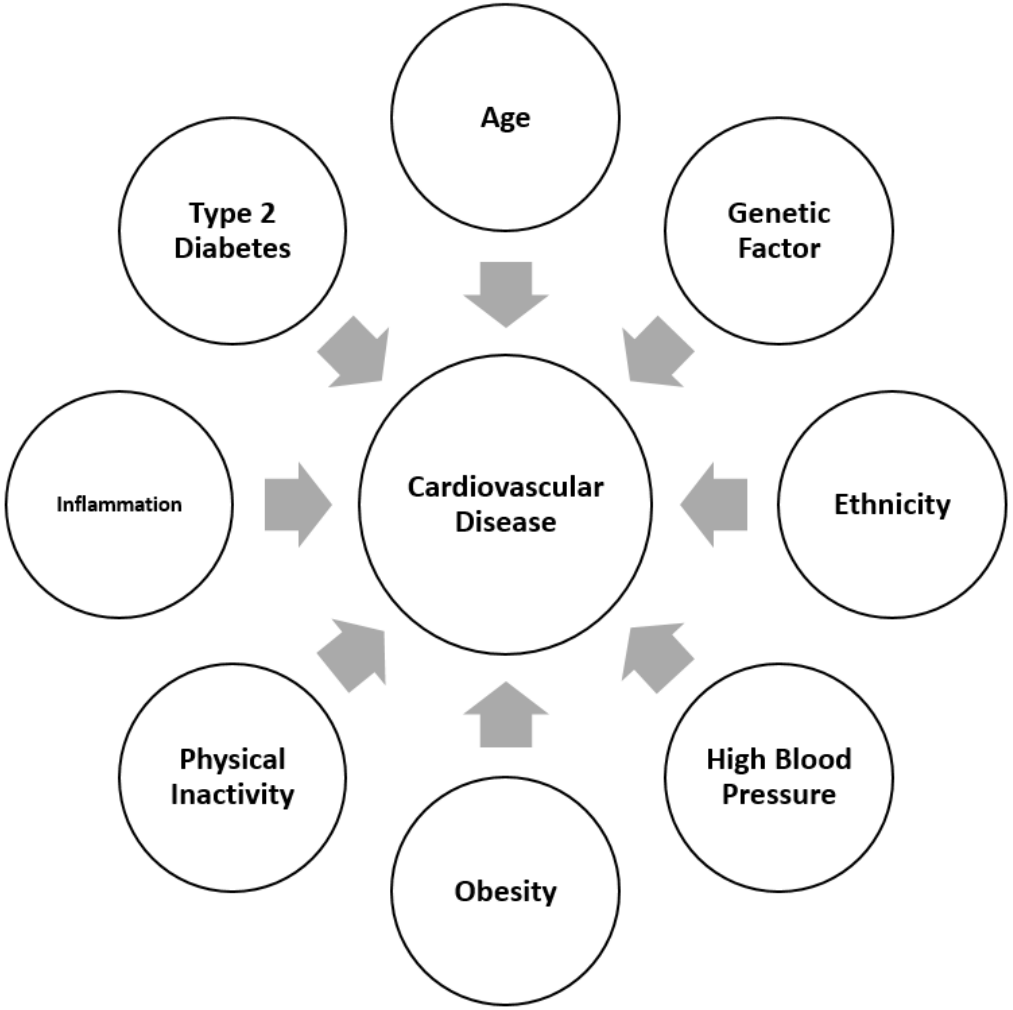

FIGURE 2.3: Risk Factors for CVD (Cardiovascular Disease).

2.4 Diabetes Mellitus

DM, CVD and obesity together is rapidly growing and has turned into a major public health issue associated with immoderate morbidity and mortality. A low-level chronic inflammation state shown clear association in insulin resistance and diabetes mellitus[34]–[36]; but also current data seems to suggest that elevation of certain cytokines vary according to ethnicity [37]. It is now apparent that a series of intracellular signaling pathways activated by a state of chronic, low-grade inflammation, particularly within the white adipose tissue, participate in the regulation of insulin signaling that in turn regulates a series of downstream signaling events [38], [39]. Moreover, inhibition of these signaling steps is known to be a primary mechanism through which inflammatory signaling leads to insulin resistance [40]–[42]. Activation of these kinases, in obesity, not only has been shown to highlight the overlap that exists between metabolic and immune pathways [44].

FIGURE 2.4: Risk Factors for Diabetes.

2.5 Psychiatric Disorder

Depression and anxiety can be induced by inflammation in peripheral and central nervous system. Variety of prostaglandins and pro-inflammatory cytokines in response to immune system [47]. Immune system is stimulated and inhibited by one of the most important type of cell white blood cell known as macrophages [44]. Pro-inflammatory cytokines secrets tumor necrosis factor alpha (TNF-a) or interleukin-6 (IL-6), and anti-inflammatory cytokine such as (IL-10) which effect immune system [48]. In turn CRPs are produced in liver stimulated by IL-6. These are the inflammatory mediator molecules communicates other army and cell of immune system such as B and T cells in adaptive immune cells [58]. There are three cornerstones linking inflammation and depression are in table 2.3

FIGURE 2.5: Symptoms of Depression.

Table 2.3: Indicators for Anxiety

S. No	Symptoms
1	Feeling nervous, restless or tense.
2	Having a sense of impending danger, panic or doom.
3	Having an increased heart rate.
4	Breathing rapidly (hyperventilation)
5	Sweating.
6	Trembling.
7	Feeling weak or tired.
8	Trouble concentrating or thinking about anything other than the present worry.
9	Having trouble sleeping
10	Experiencing gastrointestinal (GI) problems
11	Having difficulty controlling worry
12	Having the urge to avoid things that trigger anxiety

Table 2.4: Cornerstones Linking Inflammation and Depression.

S. No	Cornerstones	Reference
1	Depression risk increases when inflammation and somatic diseases comprises inflammatory process.	[46]–[49]
2	Pro-inflammatory markers levels increases in depressed individuals.	[48]
3	Depressive symptoms induced by Pro-inflammatory agents. Which can be treated with antidepressants.	[47]

Chapter 3

Materials and Methods

3.1 Methodology and Techniques

3.1.1 Selection of Location

Kahuta is the multiethnic region people from all over the Pakistan are resident of this region Kahuta is located at the distance of 25 Km from Rawalpindi and Islamabad. According to recent census in 2017 total population was 220576 [50]. This study was conducted over a period of six months from January 2018 to July 2018.

3.1.2 Ethical Approval

Ethical approval for the research study was obtained from bioethical review committees of Department of Bioscience, Capital university of Science and technology Islamabad. Questioners were designed by reviewing literature and consulting Gmeresearch and Dr. Javed Clinic

3.1.3 Inclusion and Exclusion Criteria

The inclusion criteria were as follow:

- Healthy individuals age 18 and above living in Kahuta region.

The exclusion criteria were as follow:

- Pregnant cases
- Physical injury or infections

3.1.4 Sampling and Testing Equipment

CRP detection kit, blood sugar reagent kit, cholesterol reagent kit, syringes, alcohol swabs, facemasks, gloves, Vacationer (Red, Purple and Gray Cap), BP apparatus, Stethoscope, Measuring Tape, Biochemistry Analyzer, Glucose meter kit, Test tubes, Micropipettes, Micropipettes tips (Blue and Yellow), Water Bath, Centrifuge Machine.

3.1.5 Sample Size Calculation

In order to calculate the prevalence and correlation at regional level, following formula was used to calculated the overall sample size.

$$\text{Sample Size} = \frac{Z^2 * p\,(1-p)/e^2}{Z^2 * p\,(1-p)/e^2 N} \qquad \text{Equation: 1}$$

N is the population size, e is margin error and Z is the z-score the number of standard deviations. An estimated sample size of 382 subjects was calculated using the above formula with 5% margin error, 95% level of confidence and 1.96 z-score.

3.1.6 Weight Measurement

An analogue weight measuring device was used to measure weights of subjects before measuring weight machine was calibrated and 0 error was removed by adjusting pointer to exactly zero. Weight measuring machine was placed on hard surface. Subjects were asked to remove extra clothing's like heavy jackets, shawls, and shoes. They were asked to stand straight on both feet so that equal force was applied on machine. Readings were taken twice and mean was calculated for each subjects. Measured weight was recorded on each questionnaire in Kilograms.

3.1.7 Height Measurement

10-meter rod was purchased and fixed straight with wall from floor subjects were asked to remove shoes and high heels in case of female patient. They were asked to stand straight with face direction not too lower or high. A steel ruler was used to press hairs of subjects and note down the exact height in inches. Height was recorded on questionnaire of each subject.

3.1.8 Body Mass Index Calculation

BMI was calculated using formula Kg/m^2 in Microsoft excel registered version. Subjects were classified in to six categories such as underweight, normal, overweight, obese class 1, obese class 2, and obese class 3.

3.1.9 Blood Pressure

BP was measured by using blood pressure measuring device. Device was calibrated with manual mercury based BP device with consultation of doctor. No stethoscope was required for digital measuring of BP. Subjects were asked to site calm and take rest for five minutes. Two readings were obtained and average was calculated and recorded on questionnaire of each subjects.

3.1.10 Waist Circumference

Waist circumference was measured with an anthropometric tape applied horizontally at a level laterally midway between the iliac crest and the lowest lateral portion of the rib cage and anteriorly the umbilicus.

3.1.11 Blood Sampling

Each Subject was asked to sit relaxed, suitable site for vein puncture to collect blood, by tiding the tourniquet 3 to 4 inches above was selected for insertion of syringe on the subject arm or back side of Hand. After putting gloves vein was palpated. Vein was selected, cleaned in a circular motion, after the area was cleaned, it was touched or palpated again. Subjects were asked to make a fist and avoid pumping the fist. Patient's arm was firmly gripped using thumb to draw the skin stretched and anchor the vein. Needle was inserted into the lumen of the vein. There should be an angle of 15-30 degree with the arm surface, Syringe was filled for 5CC blood. Tourniquet was removed first than needle from the patient's arm was removed using a swift and backward motion. Alcohol swab was placed immediately on the puncture site and patient was asked to apply adequate pressure to avoid formation of a hematoma. After holding pressure for 1-2 minutes. 5ml blood from each subject was collected in 5 CC Syringe. 3 ml blood was stored in red capped clot activator vacutainers for cholesterol and CRP test and 2 ml Blood was stored in grey capped vacutainer containing sodium fluoride and potassium oxalate for Glucose test.

3.1.12 Sample Preparation

Blood samples collected in red and gray capped vacationer centrifuged at 800 rpm for 5-10 min to separate serum. Using micropipette 100 micro litters of serum and cholesterol and glucose reagent poured and mixed in different disposable test tubes. Samples were then incubated in water bath for 5-10 min.

3.1.13 Biochemical Analysis

Three biochemical test were performed on blood samples.

3.1.13.1 Total Cholesterol and Blood Glucose

Prepared samples were analyzed to measure total cholesterol and blood glucose levels by Microlab 400 with ELITech Group reagents.

3.1.13.2 C - Reactive Protein Detection

To detect the presence of CRP in blood reagents and serum sample were kept at room temperature and mixed latex reagent gently prior to use. First placed 1 drop of Serum, Positive control and Negative control on separate reaction circle on glass slide. Then added 1 drop of CRP latex reagent to each of the circles. Mixed with separate mixing sticks and spread the fluid over the entire area of the cell. Then tilted the slide back and forth slowly for 2 minutes observing preferably under artificial light. Appearance of visible agglutination indicated presence of CRP in serum samples.

3.1.14 Statistical Analysis

The statistical analysis was done by using SPSS. Prevalence in the form of percentage, Pie charts and bar charts are plotted using MS Excel. Chi-square test was used to determine significant association of variable with each other using following formula.

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i} \qquad \text{Equation: 2}$$

Correlation was calculated to determine association of inflammation with obesity and its co-morbidities. Odd ratios and relative risk were calculated to determine exposure of odds outcome. Significance is defined as p-value 0.05.

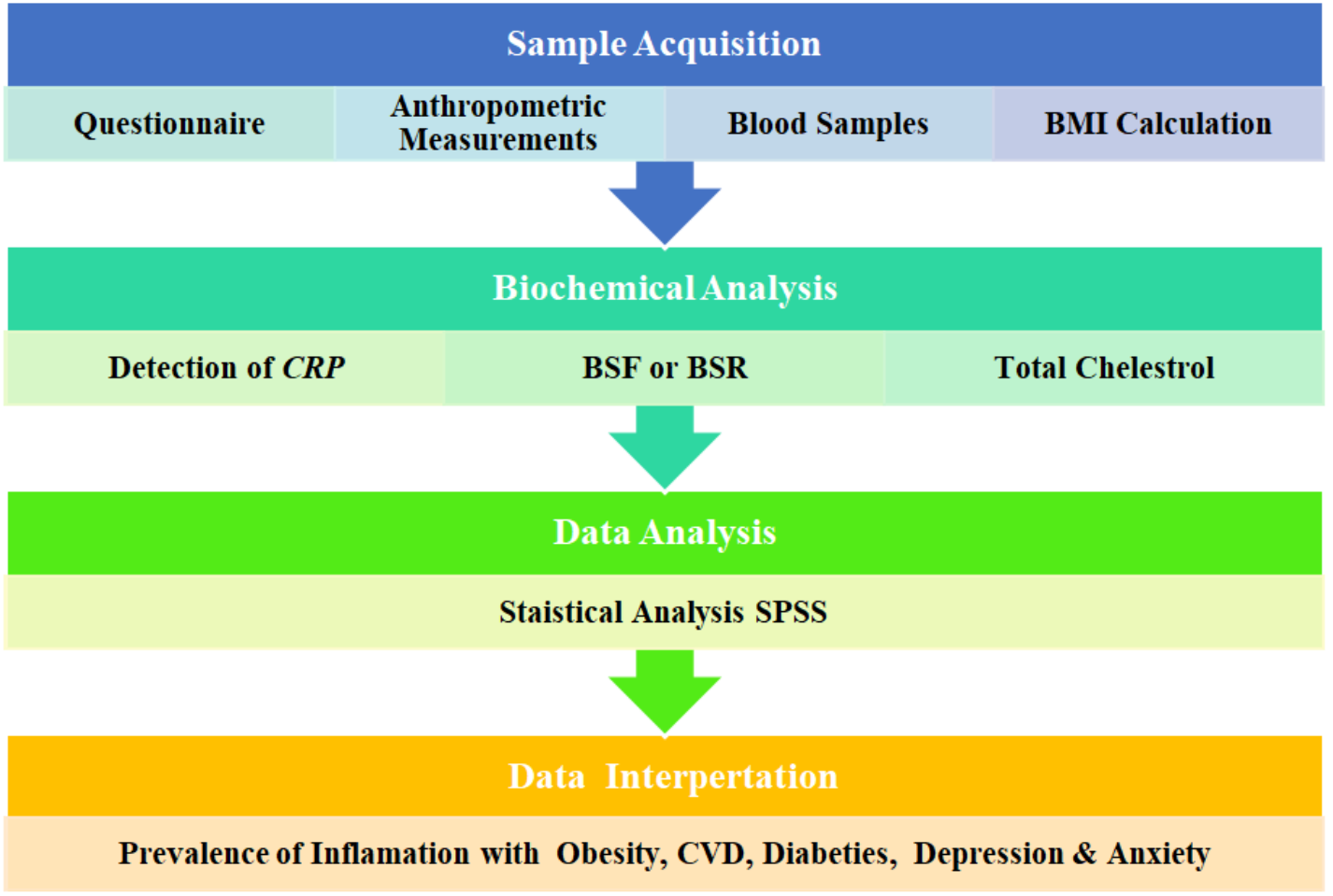

FIGURE 3.1: Overview of Research Methodology

Chapter 4

Results and Discussion

Total of 400 subject 381 subjects 50% Males and 50% Females (Figure 4.1) were selected for study.

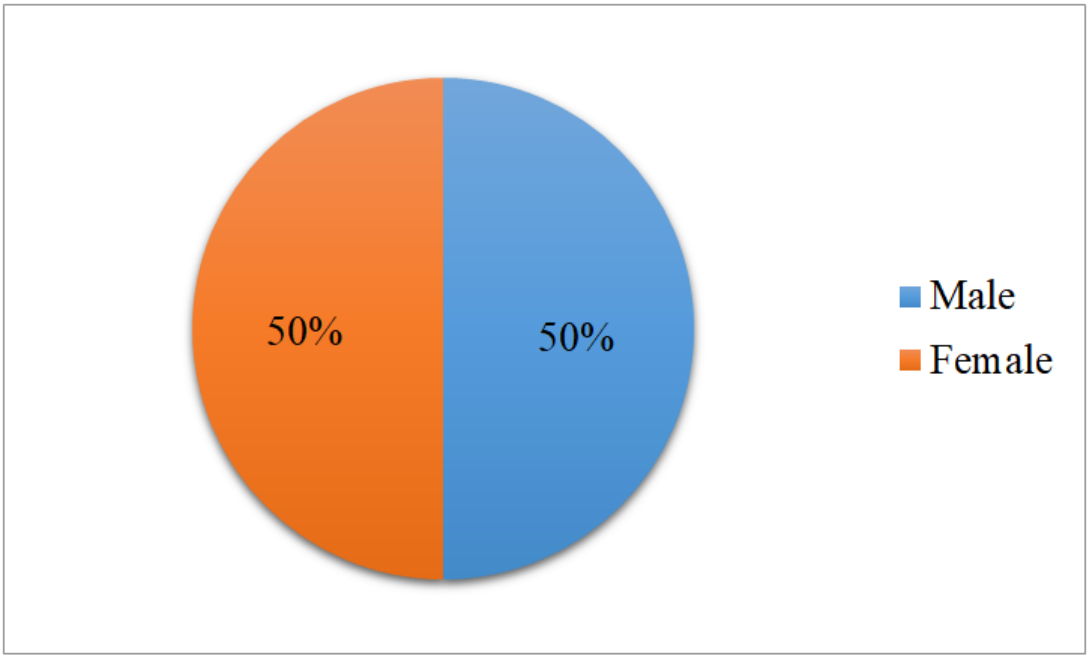

FIGURE 4.1: Percentage of Male/Female Subjects.

Respondents were divided in 3 age groups teen age (18 to 19), young adults (20 to 35) and adults (35 above). Total of 381 subjects 7% were teen age, 16% subjects were young adults, and 77% subjects were adults (Figure 4.2).

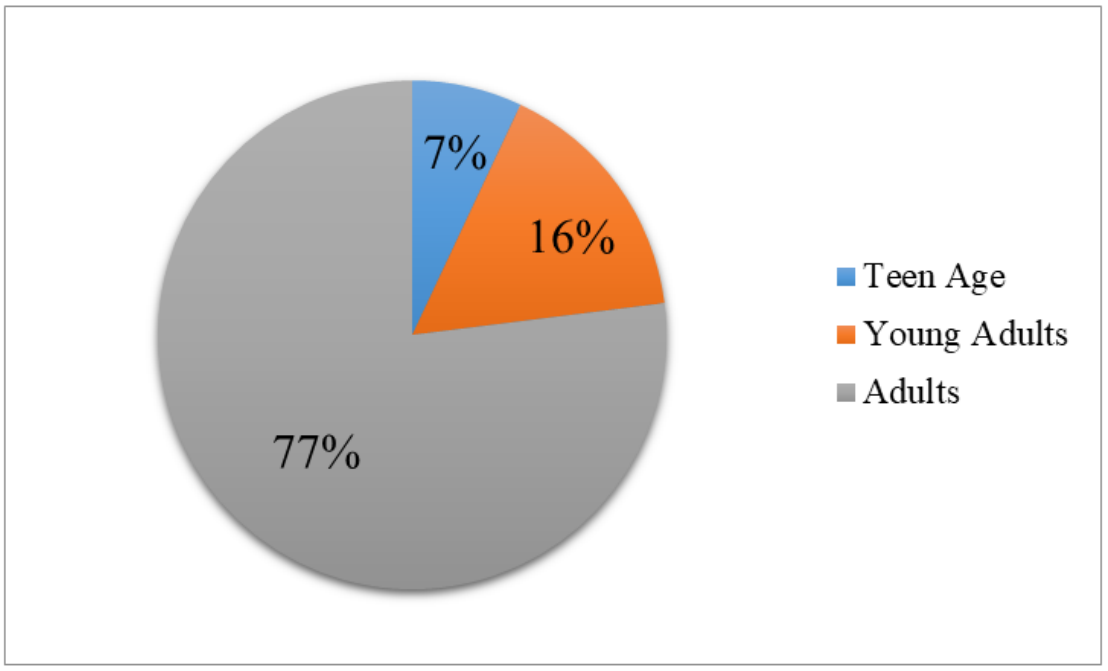

FIGURE 4.2: Age Distribution of Subject Teen Age (18 to 19), Young Adults (20 to 35) and Adults (35 above).

4.1 Prevalence of Obesity

In kahuta region prevalence of obesity is 26% (Figure 4.3). According to WHO BMI classification criteria (Table 4.1) 6% subjects were underweight(BMI >18), 40% individuals were normal weight (BMI >18 to 24.9,) 28% were overweight (BMI >25 to 29.9), 15% subjects were categorized as Obese Class I as there BMI was above 25, 8% subjects were under Obese Class II and 3% were categorized under Obese class III as there BMI was greater than 40. shown in figure 4.4 All individual having BMI greater than 30 were considered Obese and the prevalence of obesity was observed 26% in subjects of Kahuta region shown in figure 4.3.

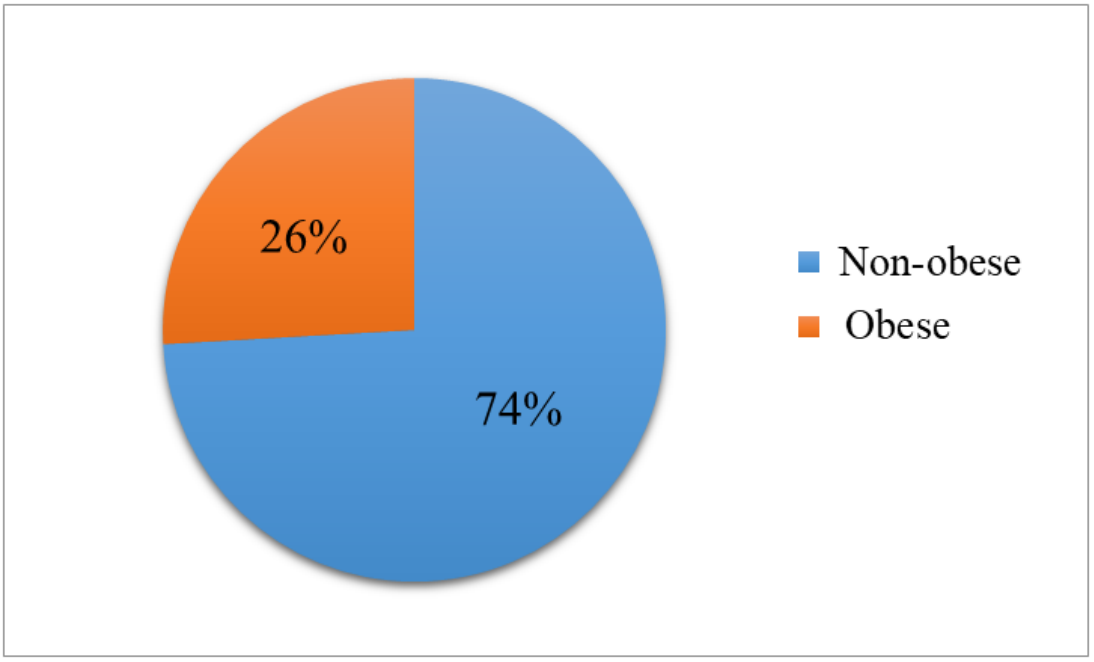

FIGURE 4.3: Prevalence of Obesity in Total Sample Collected.

TABLE 4.1: WHO BMI Classification for Normal, Overweight& Obese Individuals.

BMI	Condition
>18-24.9 Kg/m²	Normal Weight
>25-29.9 Kg/m²	Overweight
>30 Kg/m²	Obese
>30-34.9 Kg/m²	Obesity Class I
>35-39.9 Kg/m²	Obesity Class II
>40 Kg/m² or >35 Kg/m²	Obesity Class III

Prevalence of obesity in males and females shown in figure 4.5. Prevalence of obesity in female is 46% which is grater then males 26%. As compared to males females are more obese.

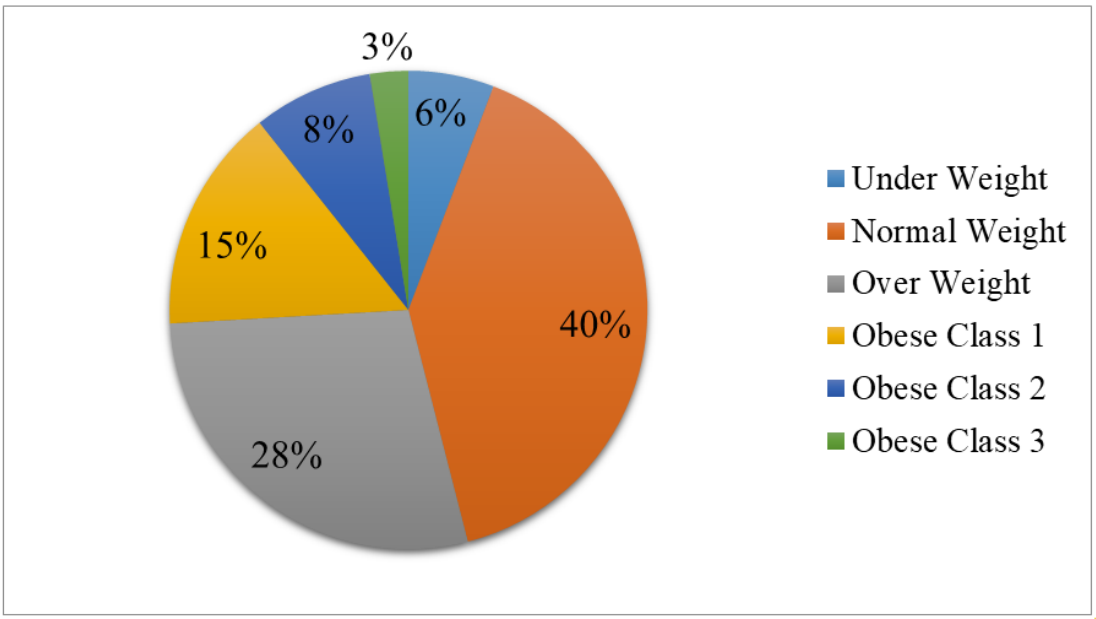

FIGURE 4.4: Prevalence of Obesity in Total Sample Collected.

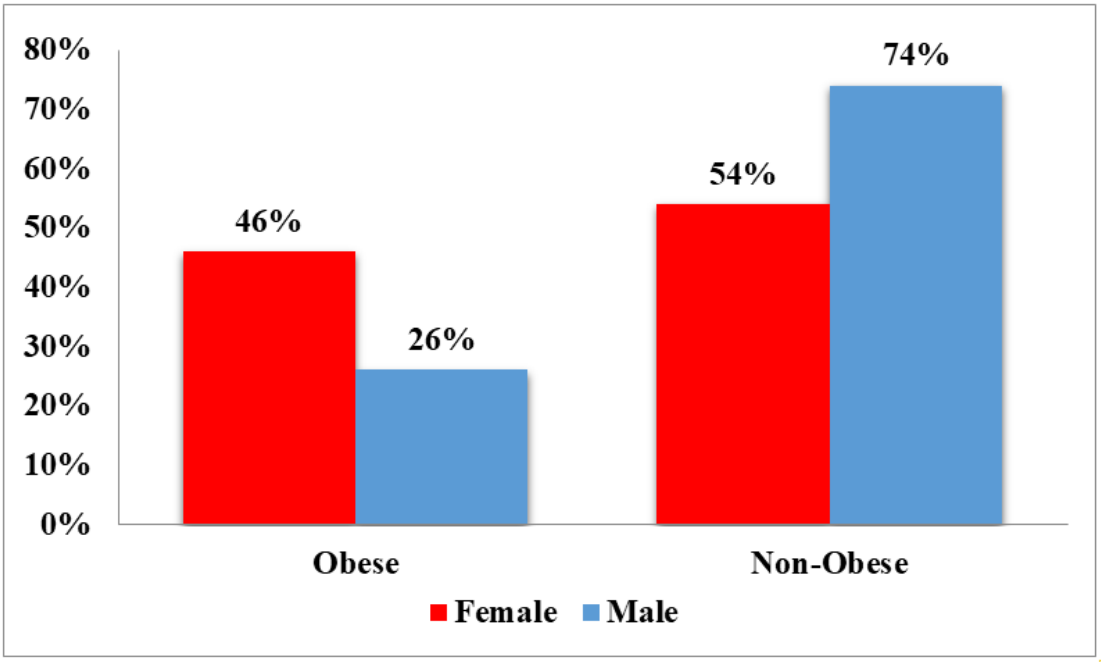

FIGURE 4.5: Prevalence of Obesity in Male and Female.

4.2 Prevalence of Inflammatory Response in Obese and Non-obese

In kahuta region 38% of obese individual and 62% non-obese individuals shown positive results for CRP inflammation marker, 20% obese individuals and 80%

non-obese individuals shown Negative results for CRP inflammation marker. As compared to non-obese subject prevalence of obese subjects having inflammation is higher than the obese subject without inflammation (Figure 4.6).

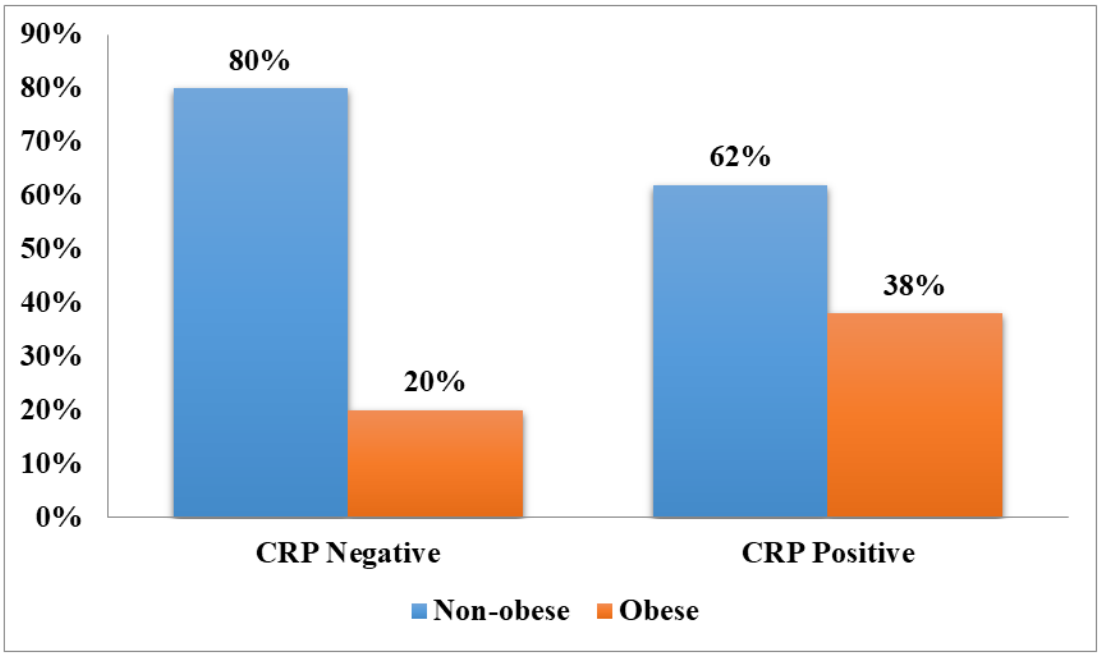

FIGURE 4.6: Prevalence of Inflammatory Response in Obese Individuals in Comparison with Non-obese CRP (C-Reactive Protien Inflammation Marker).

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Chi-square test shown positive association between obesity and inflammation with the 14.065 value at 1 degree of freedom and 0.0017 p-value. Odds ratio for obese and non-obese of developing chronic inflammation in 2.432 which represent high risk of developing inflammation in obese individuals.

4.3 Prevalence of Diabetes

Overall prevalence of diabetes in kahuta region is 14% and 86% are non-diabetic showen in figure 4.7.

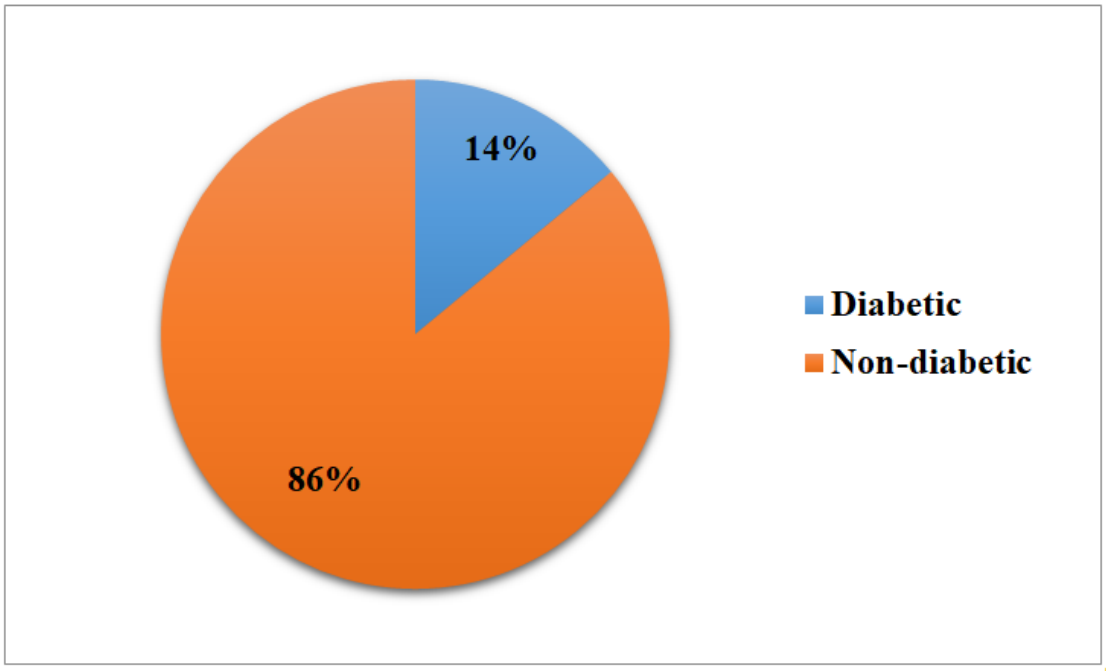

FIGURE 4.7: Prevalence of Diabetes.

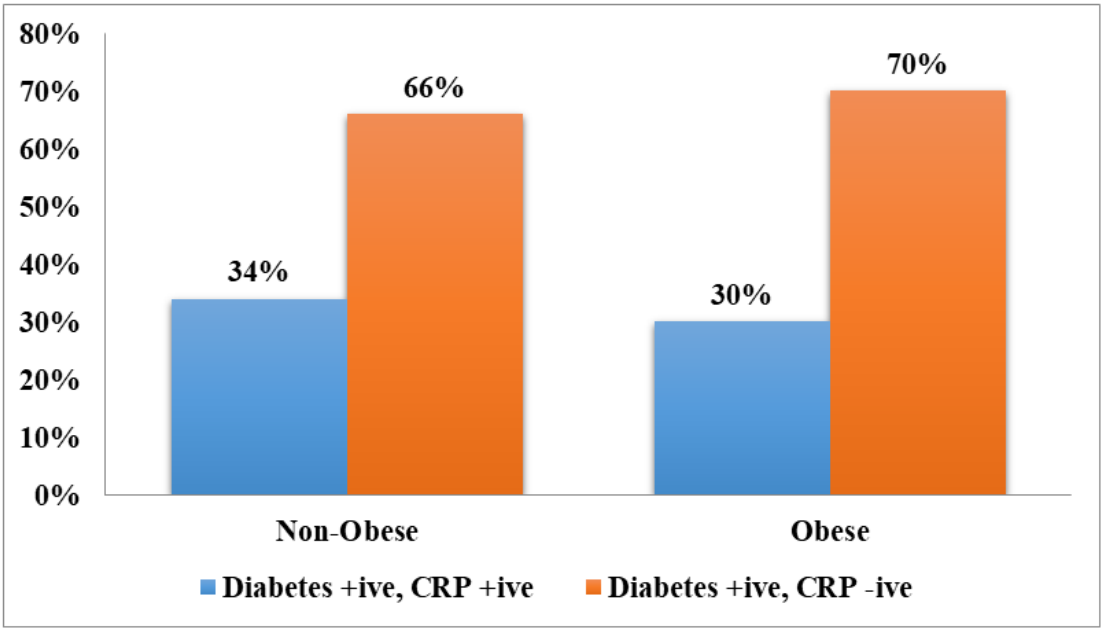

FIGURE 4.8: Prevalence of Diabetes in Obese/Non-obese.

Prevalence of Obese diabetic individuals having negative CRP inflammation marker is 70%, Non-diabetic obese individuals having positive CRP inflammation marker is 30%, Non-obese diabetic with positive CRP inflammation is 34% and Non-diabetic and non-obese with positive CRP inflammation marker is 66% (Figure 4.8).

Chi-square test shown 0.051 value at 1 degree of freedom with 0.822 p-value which is not significant association. Odds ratio for the diabetic obese is 0.857 which is lower risk.

4.4 Prevalence of Cardiovascular Diseases

Prevalence of CVD in Kahuta region is 35%. Total of 381 subject 245 individuals were CVD patients (Table 4.9).

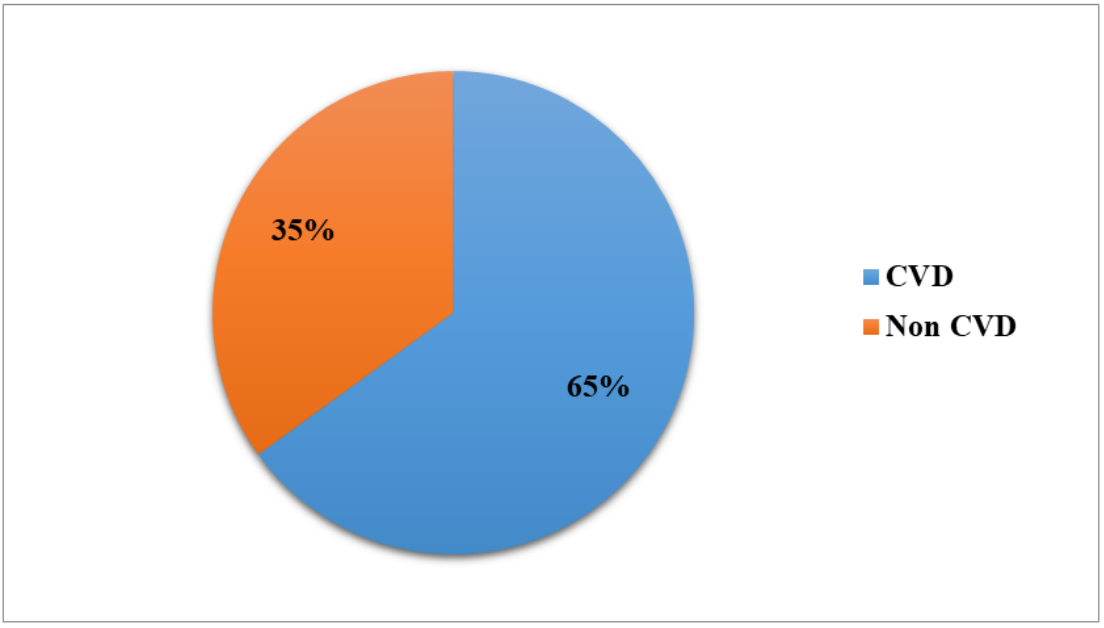

FIGURE 4.9: Prevalence of Cardiovascular Disease.

Prevalence of cardiovascular disease in obese subjects with positive CRP is 53% and with negative CRP is 47% and in Non-obese with negative CRP is 66% and with positive CRP is 34%.

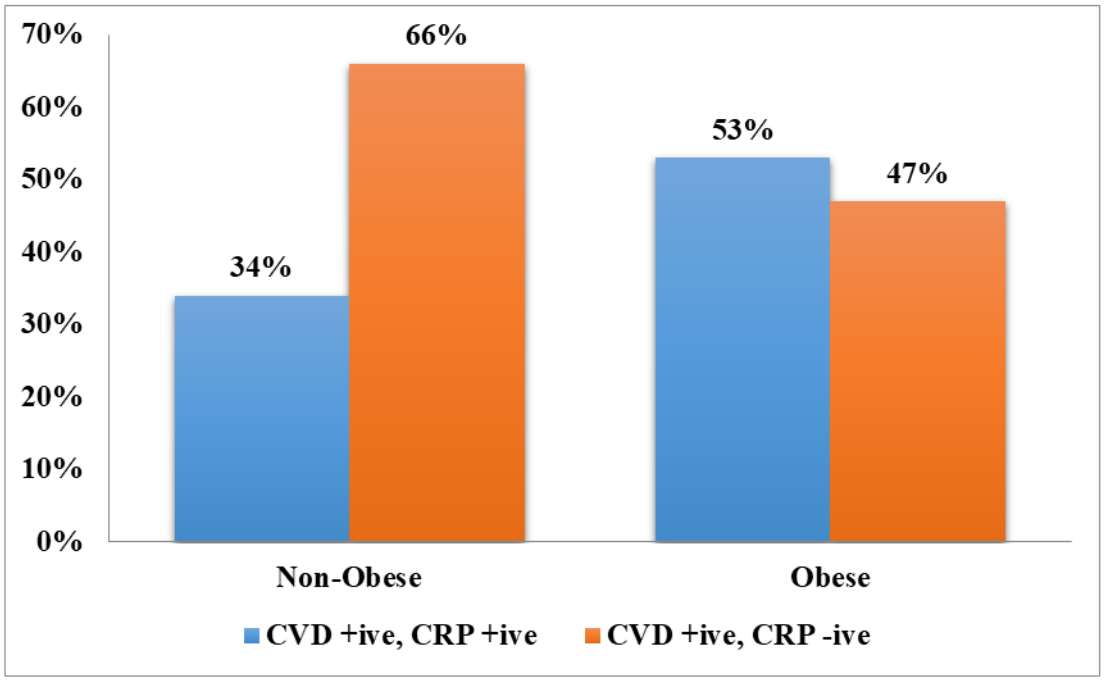

FIGURE 4.10: Prevalence of Inflammation Associated Cardiovascular Disease. CVD (Cardiovascular Disease), CRP (Inflammation Marker), +ive (Positive), -ive (Negative)

Chi-square test shown 7.806 value and 1 degree of freedom at 0.05 p-value which is significant association. Odds ratio for cardiovascular disease in obese and non-obese is 2.190 which represent higher risk of developing cardiovascular disease in obese subjects.

4.5 Prevalence of Hypertension

Hypertension is most common CVD observed in Kahuta region. Prevalence of hypertension is 8%. 26% high blood pressure and 66% were normal (Table 4.11).

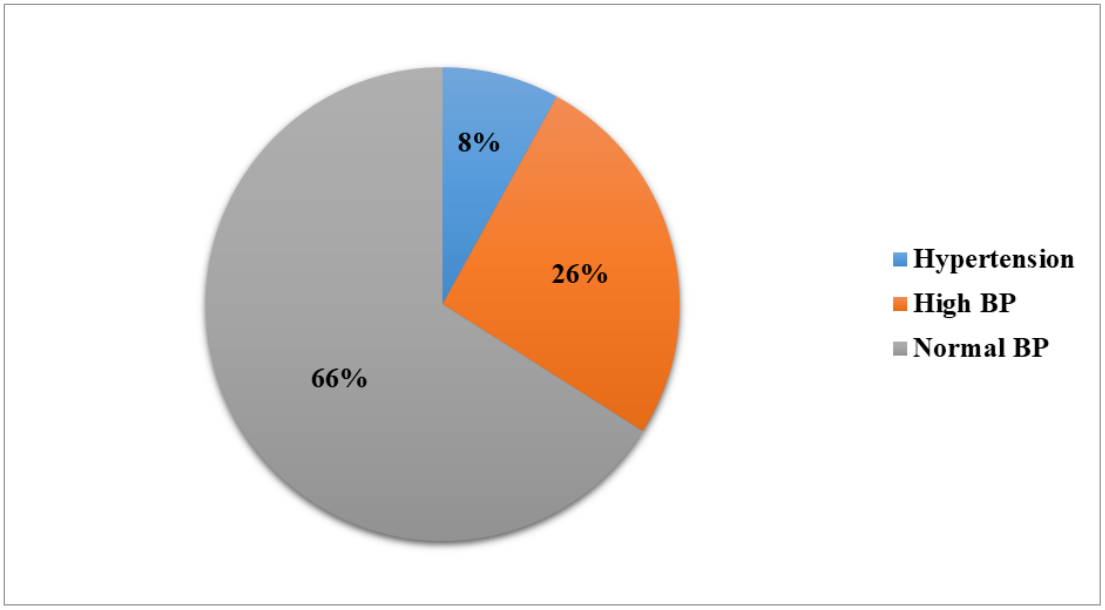

FIGURE 4.11: Prevalence of Hypertension.

Prevalence of hypertension in non-obese with positive CRP is 47%, with negative CRP is 53%, and prevalence of hypertension in obese subject with positive CRP is 30% amd with negative CRP is 70%. Chi-Square test shown significant association with 8.97 value with 1 degree of freedom at 0.03 P-Value. Odds ratio for hypertension in obese and non-obese is 0.476 which represents low risk of hypertension.

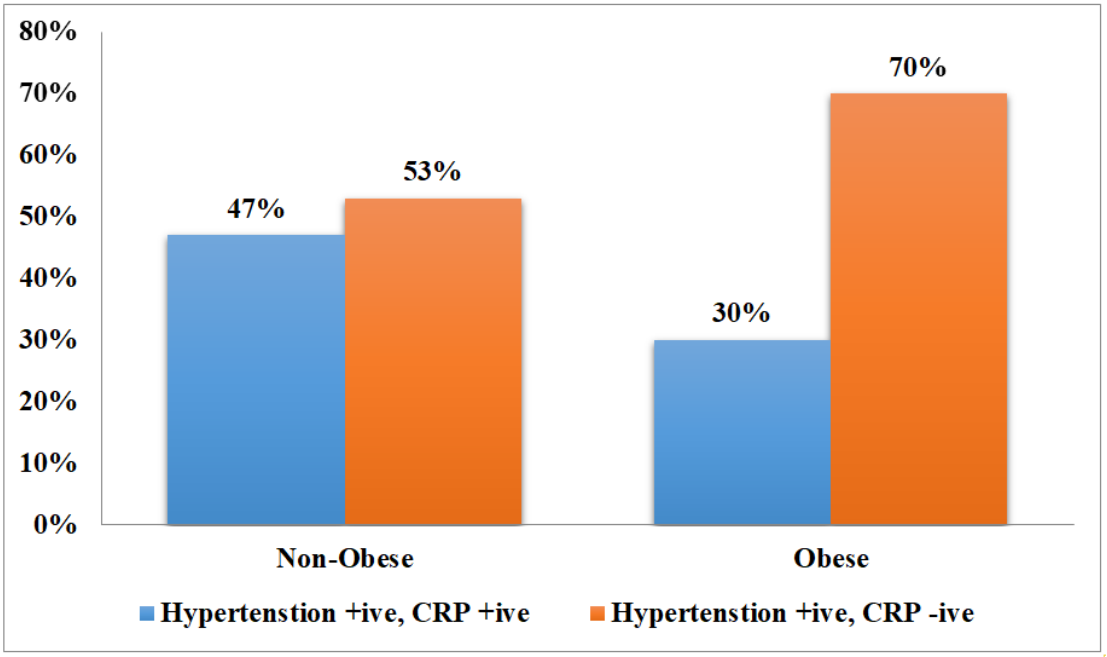

FIGURE 4.12: Prevalence of Inflammation Associated Hypertension in Obese and Non-obese Subjects. CRP (Inflammation Marker), +ive (Positive), -ive (Negative).

4.6 Prevalence of Anxiety

Overall prevalence of anxiety in subjects of Kahuta region is 55% and 45% were normal (Figure 4.13).

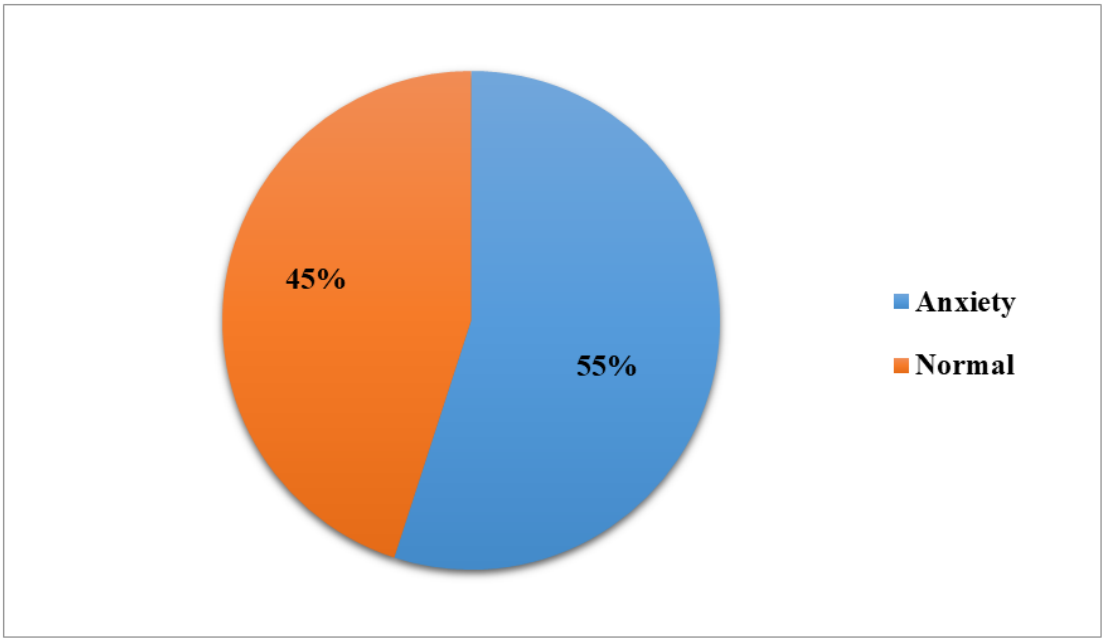

FIGURE 4.13: Prevalence of Anxiety.

Prevalence of anxiety in non-0obese with positive CRP is 33% and with negative CRP is 77%. Prevalence of anxiety in obese with positive CRP is 55% and with negative CRP is 45%. Chi-square test shown 7.89 value and 1 degree of freedom at 0.05 p-value which represents significant association. Odds ratio for anxiety in obese and non-obese is 2.482 which represents greater risk for anxiety (Figure 4.14).

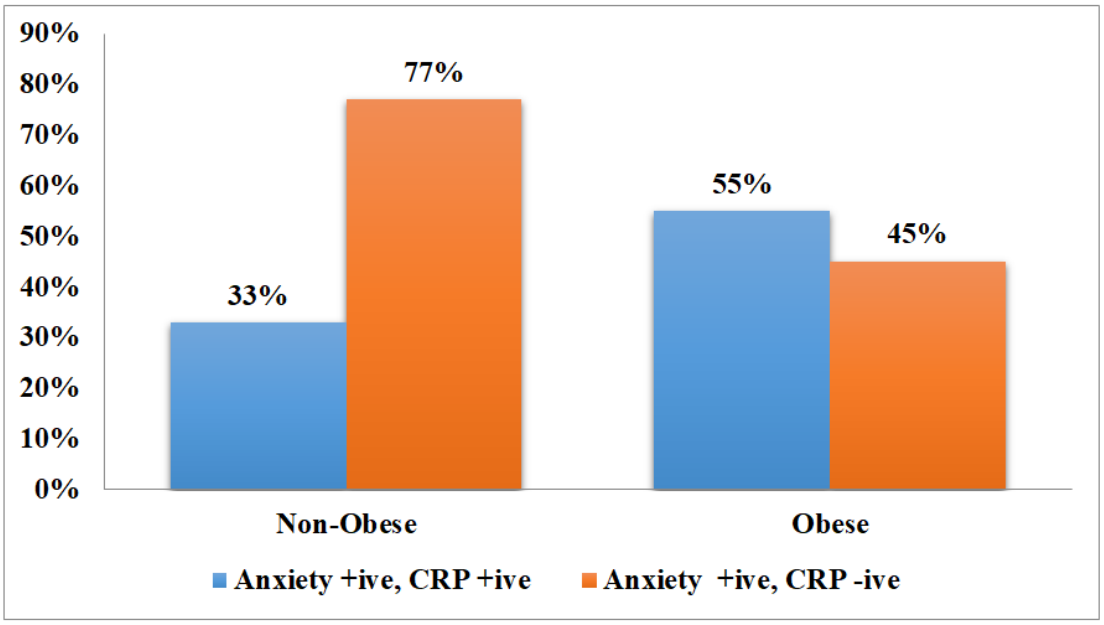

FIGURE 4.14: Prevalence of Anxiety CRP (Inflammation Marker), +ive (Positive), -ive (Negative).

4.7 Prevalence of Depression

Prevalence of depression is 39% 61% were normal in kahuta region (Figure 4.15) Prevalence of depression in non-obese with positive CRP is 36% , with negative

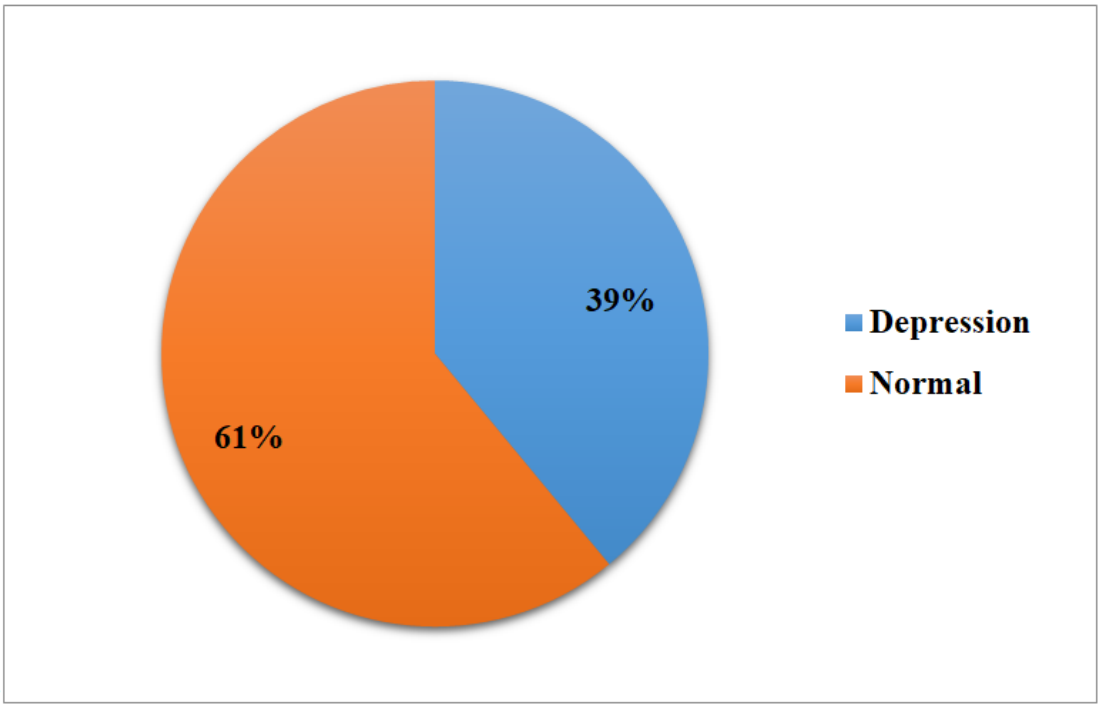

FIGURE 4.15: Prevalence of Depression.

CRP 64% and in obese with positive CRP 50%, with negative CRP is also 50%. Chi-Square test shown 2.148 value with 1 degree of freedon at 0.143 p-value which represents non-significant association.

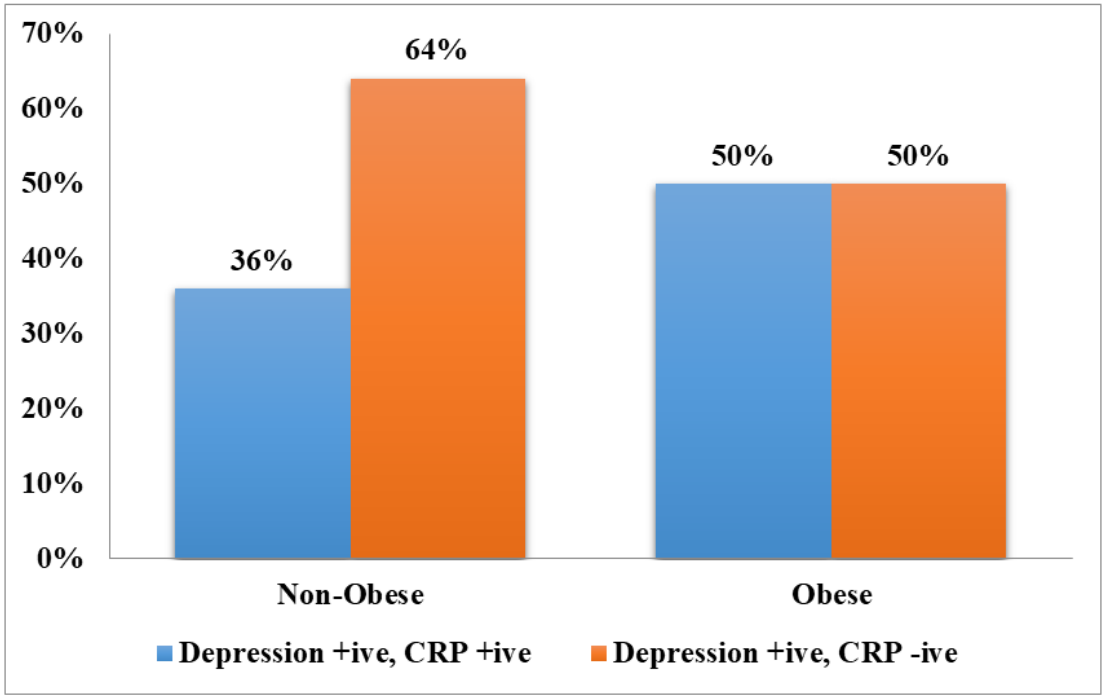

FIGURE 4.16: Prevalence of Depression CRP (Inflammation Marker), +ive (Positive), -ive (Negative).

In this study total 381 subjects 190 (49.9%) males and 191 (50.1%) females included. Mean age of over all subjects was 41.71 years with maximum number of subjects was 40 years. BMI mean value was 26.4. Mean values of calculated BMI suggests that peoples of Kahuta region are Overweight and are not Obese, systolic diastolic blood pressure is observed Normal, Blood Random Sugar levels were observed above them Normal. This might be because of high consumption of soft drinks, juices, tea and sweets as well as due to low physical activity (walk, exercise etc.) observed other than workload. Respondents were divided in 3 age groups teen age, young adults and adults. Total of 381 subjects 28 (7.3%) were teen age, 60 subjects (15.7%) were young adults, and 293 subjects (76.9%) were adults.

According to WHO BMI classification criteria 6% subjects were underweight, 40% individuals were normal weight 28% were overweight, 15% subjects were categorized as Obese Class I as there BMI was above 25, 8% subjects were under Obese Class II and 3% were categorized under Obese class III as there BMI was greater than 40. All individual having BMI greater than 30 were considered Obese and the

prevalence of obesity was observed 26 % in subjects of Kahuta region. Risk of obesity in Kahuta region is observed 1.530 time grater and 0.564 odds ratio in male and female with 95% confidence interval. Odds ratio of inflammation mediated obesity is 2.432 and risk estimate is 0.528 time greater with 95% confidence interval. It is observed that obesity and inflammation are significant positively correlated with each other at 0.05 P-value level. In this study no significant association found between obesity, diabetes and inflammation. Koca TT (2017) reported significant positive correlation between inflammation, obesity and diabetes.

Prevalence of CVD in Kahuta region is 35%. Total of 381 subject 245 individuals were CVD patients. Hypertension is most common CVD observer in Kahuta region. Prevalence of systolic and diastolic hypertension is 8%. Odds ratio of male and female for CVD is 0.147 and risk of CVD in males is 1.507 time greater and in female 0.629 times greater. CVD is moderately strong associated with BMI. Odds ratio for inflammation mediated CVD is 2.012 and the risk estimate is 0.796 times greater in Kahuta region for CVD. Total of 246 CVD subjects 41 individual were diabetic and 205 individuals were non-diabetic. It is observed that CVD and inflammation is significant positively correlated with each other at 0.01 p-value level. CVD and obesity are significant positively correlated with each other at 0.05 p-value. Phi and Cramer's V test suggesting week association between obesity and CVD. SAH Bokhari (2015) reported significant association of CVD with inflammation in South-Asian Population from 2001- 2012.

Overall prevalence of anxiety in subjects were 55% total of 381 209 subject were anxiety patients and 172 were normal. Prevalence of depression is 39% total of 381 subject 149 were patients of depression 232 were. Total of 381 subject 121 females and 88 males were anxiety patients included in table Odds ratio for anxiety is 0.499. Risk of anxiety in females is 0.703 times greater and in males 1.408 times greater. In non-obese subject 158 individuals were anxiety patients, 124 were normal, 51 obese subjects were anxiety patients, and 48 were normal obese subjects. In kahuta region spps correlation test suggested week association between

inflammation and anxiety but negative correlation with depression. Alvaro Commacho (2013) reported involvement of different inflammatory mediator molecules in causing symptoms of anxiety and depression.

Chapter 5

Conclusions and Recommendations

Prevalence of obesity in Kahuta region is higher in females as compared to male. Obesity induces inflammatory responses in obese individuals. Significant association found between obesity and inflammation at 0.001 p value. Association of cardiovascular diseases with obesity and inflammation is significant at 0.05 p-value. Chi square test shown 0.8 p-value association of diabetes with obesity and inflammation is not significant.

There is need to create awareness among the peoples of region about the risk factor of inflammatory disorders i.e. unhealthy diet and lack of physical activity. This task can be achieved by print, electronic media community elders, religious leader, health workers. Education department can play important role by organizing event of healthy activity to make people aware and adoption of healthy life styles.

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Appendix A

QUESTIONNAIRE FOR RESEARCH PROJECT

PID No:

Project Title: The Prevalence of Cardiovascular, Psychiatric and GI disorders *in* Obese Subjects of Rawalpindi

Investigator(s): Capital University of Science & Technology, Expressway, Kahuta Road, Zone-V, Islamabad. PHONES: +92-51-2512800-1, +92-51-4486700-4, FAX NUMBER: +92-51-4486705 UAN: +92-51-111-555-666 Extensions: 123,280,0

Instructions:

1. This survey form may contain words that are new to you. If you read any words that are not clear to you, please ask the person who gave you this form to explain them to you.
2. Your records will be kept confidential and will not be released without your consent except as required by law.
3. Your identity will be kept private.
4. If the results of this study are written in a scientific journal or presented at a scientific meeting, your name will not be used.
5. Your initials _____ indicate your permission to be identified by name in any publications or presentations.
6. If you do not want to be acknowledged by name in any publications or presentations, please initial here _____.
7. The data will be stored in a locked file cabinet.
8. Your signed consent form will be stored in a cabinet separate from the data.
9. Your decision to take part in this research study is entirely voluntary.
10. You may refuse to take part in or you may withdraw from the study at any time without penalty or loss of benefits to which you are normally entitled.
11. You may be asked to leave the study for any of the following reasons:
12. Failure to follow the Project Director's instructions;
13. A serious adverse reaction which may require evaluation;
14. The Project Director thinks it is in the best interest of your health and welfare; or
15. The study is terminated.
16. You may wish to discuss this with others before you agree to take part in this study.
17. If you have any questions about the research now or during the study, please contact:

_____

QUESTIONNAIRE FOR RESEARCH PROJECT

GMEResearch

PID No:

BIODATA: (This information provided by Patient will be confidencial)

First Name: _____ **Mid Name:** _____ **Last Name:** _____

Date of Birth _____ **Age:** _____ **Gender:** _____ **Contact No: (Office)** _____

Home: _____ **Cell:** _____ **Email:** _____

Permenant Address:

Address: _____

City: _____ **Province:** _____

Temporary Address:

Address: _____

City: _____ **Province:** _____

1. ANTHROPOMETRIC MEASUREMENT

Weight (kg)	
Height (m)	
BMI (kg/m^2)	
Blood Sugar mmol/L	
Total cholesterol (TC)	
Triglycerides (TG) (mmol/l)	
HDL-C (mmol/l)	
LDL-C (mmol/l)	
CRP	
Myoglobin	
CK-MB	

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP C Reactive Protein; CK-MB, Creatine Kinase-MB.

2. OBESITY, CVD, GI and PSYCHIATRIC RELATED COMPLAINTS

High blood pressure	Yes	No	Diabetes	Yes	No
CVD	Yes	No	Eating disorder	Yes	No
Difficulty in stool passing	Yes	No	Depression	Yes	No
Anxiety	Yes	No	Insomia	Yes	No
Headaches	Yes	No			

3. FAMILY HISTORY

Obese Persons in family

Father	Sister	Uncle	Mother's Sister
Mother	Brother	Aunty	Mother's Brother

4. PHYSICAL ACTIVITY

Morning walk	Evening walk	Work at home	Outing

QUESTIONNAIRE FOR RESEARCH PROJECT

GMEResearch

PID No:

5. **DIETARY HISTORY**

Breakfast	Lunch	Dinner

6. **SOCIAL AND PERSONAL HISTORY**

- Education: _____
- Job: _____Part time /Full time
- Do you have children? No / Yes - How many? _____
- Marital status: Single / Married /Separated / Divorced

7. **MEDICAL/CLINICAL HISTORY**

Medication to control obesity	
Diet plan to control obesity	
Any surgery if yes when or for what	
Medicins using for any other diesease	
Smoking or counsuption of anyother tobaco product	

8. **SAMPLES**

Blood Sample:	

Thank you for completing the questionnaire please return it to_____ **Department of Health and Life Scinece, Capital University of Science and Technology, Islamabad.** If you have any concerns regarding this research please contact me or my supervisor in the first instance.

Consent

I have read the above description of this research study. I have been informed of the risks and benefits involved, and all my questions have been answered to my satisfaction. Furthermore, I have been assured that any future questions I may have will also be answered by a member of the research team. I voluntarily agree to take part in this study. I understand I will receive a copy of this consent form,

_____ Subject's Signature

_____ Date

3

Appendix B

Gender	BMI	BSR	CRP	SysBP	DiaBP	TC	CVD	Anxiety	Depression
Female	Normal weight	Normal	Positive	Normal	Normal	Normal	Yes	Yes	Yes
Female	Normal weight	High	Negative	High	Normal	High	Yes	No	No
Female	Normal weight	High	Negative	Normal	Normal	High	No	No	No
Female	Normal weight	High	Negative	Normal	Normal	Normal	No	Yes	Yes
Female	Normal weight	Normal	Positive	Normal	Hypertension	Normal	Yes	No	No
Female	Normal weight	Normal	Negative	Normal	Normal	Normal	Yes	Yes	Yes
Female	Normal weight	Normal	Positive	Hypertension	Hypertension	High	Yes	Yes	Yes
Female	Normal weight	Normal	Negative	High	Normal	Normal	Yes	No	No
Female	Normal weight	Normal	Positive	Normal	Normal	High	Yes	Yes	Yes
Female	Normal weight	High	Negative	High	High	High	Yes	No	Yes
Female	Normal weight	Normal	Positive	Normal	Normal	Normal	Yes	Yes	Yes
Female	Normal weight	Normal	Positive	Normal	Normal	Normal	Yes	No	No
Female	Normal weight	Normal	Positive	Normal	Normal	Normal	No	Yes	No
Female	Normal weight	Normal	Negative	Hypertension	High	Normal	Yes	Yes	No
Female	Normal weight	Normal	Negative	High	High	Normal	Yes	Yes	Yes
Female	Normal weight	Normal	Negative	High	Normal	High	Yes	Yes	No
Female	Normal weight	Normal	Positive	Normal	High	Normal	Yes	No	No
Female	Normal weight	High	Positive	High	Normal	Normal	Yes	Yes	Yes
Female	Normal weight	High	Positive	Hypertension	Hypertension	High	Yes	Yes	Yes
Female	Normal weight	High	Positive	High	Hypertension	High	Yes	Yes	Yes
Female	Normal weight	High	Negative	High	Normal	Normal	Yes	Yes	Yes
Female	Normal weight	High	Negative	Normal	Normal	High	Yes	Yes	Yes
Female	Normal weight	High	Negative	Normal	Normal	Normal	No	No	No
Female	Normal weight	High	Negative	Normal	Normal	Normal	No	No	No
Female	Normal weight	High	Negative	High	High	Normal	Yes	Yes	Yes
Female	Normal weight	High	Positive	High	Hypertension	Normal	Yes	Yes	Yes
Female	Normal weight	High	Negative	High	High	Normal	Yes	Yes	Yes

Appendix C

FIGURE 1: Medical Camp at Dr. Javed's Clinic

FIGURE 2: Medical Camp at Dr. Javed's Clinic

FIGURE 3: Medical Camp at Dr. Javed's Clinic

FIGURE 4: Medical Camp at Nogran Village

FIGURE 5: Prepared Samples After Centrifugation to Separate Blood Serum

FIGURE 6: Biochemical Testing at Lab

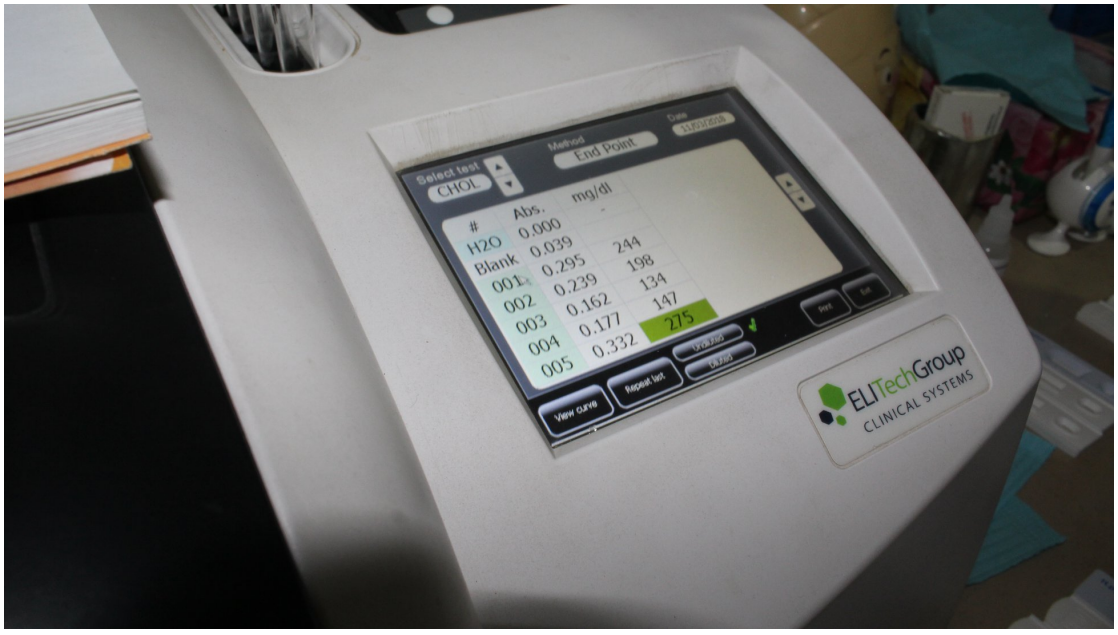

FIGURE 7: Biochemical Testing at Lab

FIGURE 8: Biochemical Testing at Lab