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Pattern and association of inflammatory markers with some cardiovascular risk factors in overweight and obese patients attending Obesity Clinic in a tertiary hospital in Oyo state.

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ABSTRACT
Background:
Obesity is associated with low grade inflammation of adipose tissue resulting from chronic activation of pro inflammatory cytokines: C reactive protein, Tumour necrosis factor alpha, Interleukin-6. These cytokines have been shown to be associated with cardiovascular risk due to their involvement in the pathogenesis of atherosclerotic vascular diseases.
Purpose :To determine the association betweencytokines:hscrp and TNF alpha with conventional cardiovascular risk factors(Diabetes mellitus, high blood pressure, low high density lipoprotein cholesterol level, high triglyceride level, high total cholesterol level)
Methods:Two hundred and seventy five obese and overweight individuals were involved in this study.They were stratified into overweight, stages 1, 2 and 3 of fifty five participants each based on body mass index, fifty five normal BMI group as control recruited from staff of the hospital and patient relatives and community residents within a four month period. Serum inflammatory markers (hs-CRP and TNF-α) were measured using enzyme linked immunosorbent assay from Span biotech limited, while TChol, HDLc, FPG, Trig were assayed using Randox commercial kit. LDLc was assayed using Cobass CII.
Results:This study revealed significant correlation between the hs-crp and the conventional cardiovascular risk factors (TChol, HDLc, LDLc, Trig., FPG) and also a similar correlation was seen with TNF alpha and the conventional cardiovascular risk factors (TChol, HDLc, LDLc, Trig., FPG.
Conclusion:There is significant association between inflammatory markers and conventional cardiovascular risk factors (Diabetes mellitus, high blood pressure, low high density lipoprotein cholesterol level, high triglyceride level, high total cholesterol level)
KEYWORDS: Cardiovascular disease risk, Inflammatory markers, Obesity, Diabetes mellitus, Hypertension, Dyslipidaemia

I. INTRODUCTION

Overweight and Obesity pose a real threat to health in children as well as in adults all over the world.[1] This includes the developed as well as the developing countries. The epidemic of obesity and its associated chronic diseases especially cardiovascular diseases is now growing in the developing world as well.[1]This significant acceleration in the incidence of obesity also indicates that low-incomecountries are now confronted with a double burden where both communicable and chronic non-communicable diseases co-exist. WHO projects a doubling by the year 2030 in mortality rates resulting from Ischaemic Heart Disease in the African region,[2]as well as a prediction by the year 2025 of the largest increase in prevalence of Diabetes Mellitus in developing countries.[2]

Obesity is anatomically characterized by an excessive adipose tissue mass.[1]Adipose tissue is not simply a storage depot for energy. Instead, it is a complex, metabolically active tissue that secretes

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a variety of signalling molecules and pro-inflammatory factors that affect systemic metabolism.[3]Obesity, which is an important risk factor for cardiovascular disease (CVD), is currently viewed as a pro-inflammatory state with an increase in the expression of inflammatory cytokines including Tumor Necrosis Factor-α (TNF-alpha) and Inter-leukin-6 (IL-6).[3]

Current knowledge of white adipose tissue acting as an endocrine organ and playing major role in the regulation of insulin sensitivity and lipid metabolism has led to the discovery of various obesity-related biomarkers (the so-called adipocytokines). The adipose tissue produces a variety of hormones and cytokines and thereby actively participates in a network of biomarkers that may be relevant for the development of cardiovascular disease.[3]The identification of these obesity associated biomarkers related to cardiovascular disease risk is important for scientific reasons to gain insight into pathophysiology, and for clinical and public health reasons. Available studies in this regard were mainly in the white population despite the increase in the prevalence of obesity and the consequent increase in cardiovascular disease in the black population,[4] Furthermore, there are racial differences in inflammation associated with obesity and insulin resistance[5]and there exist significant geographic variation in pattern of dyslipidaemia due to dietary, geographic, ethnic and socio-cultural practices.[6]

The experience at the obesity clinic of LAUTECH teaching hospital Ogbomoso also showed a large percentage of our patients presenting with one or two cardiovascular risk factors at first visit(high blood pressure, impaired glucose tolerance, high serum triglyceride level, low high density cholesterol level, high low density cholesterol level)

Materials and Methods.

This study is a descriptive cross sectional study carried out at the Chemical Pathology departmentof LAUTECH teaching hospital,Ogbomoso.

Participants were selected based on the diagnostic criteria for overweight (BMI 25-29.9kg/m^2), mild (30-34.9kg/m^2), moderate (35-39.9kg/m^2) and severe obesity (40kg/m^2) according to World Health Organization. The control group were age and sex matched individuals with BMI (20 -24.9kg/m^2) selected from the general population.

Ethical clearances were obtained from the ethical review committee of Ladoke Akintola University of Technology Teaching Hospital, Oyo State.

SAMPLE COLLECTION, PROCESSING AND LABORATORY ANALYSIS

Clinical Evaluation

Each participant had a clinical assessment of their blood pressure according to the American Heart Association guideline. The systolic and diastolic blood pressures were recorded, for each of the measurements, three readings 5minutes apart were taken and the average of the three readings was calculated to obtain the final blood pressure using a standard mercury sphygmomanometer with an appropriate cuff size. Participants were classified as hypertensive when their systolic and/or diastolic blood pressures are greater than or equal to 140 mmHg and 90 mmHg.[7] Their weight, height, abdominal circumference, waist circumference and hip circumference were measured using standard instruments.

Height measurements was taken using a stadiometer to the nearest 0.1m,with the head aligned in the Frankfort horizontal. Three measurements were taken and the average of the measurement was recorded.

A beam balance was used to weigh participants to the nearest 0.1kg. The weight wastaken in kilograms (kg). Three measurements were taken and the average of the measurement was recorded.

The waist circumference was measured at the approximate midpoint between the lower-margin of the last palpable rib and the top of the iliac crest along the mid axillary line.

The hip circumference was taken at the level of the greater trochanters with feet placed together.

For each of waist and hip circumference, three measurements to the nearest 0.1cm was taken, the average was calculated and recorded.

The following calculations were made

The Body Mass Index was calculated using the following formula[8]:

BMI = weight (kg) / [height (m)]^2

Waist-Hip ratio was calculated using the formula:

waist circumference(cm)/hip circumference(cm).

Sample Collection

After an overnight fast of about ten hours starting between 8pm and 8am, fifteen milliliters of venous blood from the cubital vein was withdrawn aseptically from the participants. Five millilitres from the withdrawn blood was aliquoted into plain bottle for serum hs-CRP and TNF α levels, five millilitres was aliquoted into 0.1% Sodium EDTA bottle for lipid profile (Total cholesterol, HDL

cholesterol, Triglyceride, LDL cholesterol), and five millilitres was aliquoted into fluoride oxalate bottle for fasting glucose estimation. Each sample was centrifuged at 3,000 rpm x g for 10 – 15 minutes using Everich centrifuge model 80-2, the centrifuge speed was routinely checked with a strobe tachometer as well as the timer according to College of American Pathologists (CAP) guidelines. The plasma and serum obtained was aliquoted into screw cap plane bottle and stored frozen at -20^{0}C , the temperature of the freezer was monitored with the daily check and recording of (National Institute of Standards and Technology)NIST-traceable thermometer immersed in the freezer until the time of analysis.

Laboratory Analysis of biochemical parameters

Fasting plasma glucose, plasma total cholesterol,plasma high density lipoprotein cholesterol, plasma triglyceride were determined spectrophotometrically using commercial kit (Randox)[9]

LDL-Cholesterol was determined by Homogenous enzymatic colorimetric assay using COBAS[10] C11. Control samples were assayed with each sample batch. Intrabatch and interbatch analysis was also carried out on the samples.

Serum hs-CRP and Serum TNF alpha were assayed usinga double-antibody sandwich Enzyme linked immunosorbent assay Kit from SPAN BIOTECH LIMITED,[11] Hong Kong.(LOT 2017111501,2017111502, 2017111503). (LOT NUMBER 2017111504, 2017111505, 2017111506).The assays were done using Labtech LT-4000 microplate Reader at a wavelength of 450nm.

II. STATISTICAL ANALYSIS

Data was analyzed using the Statistical Package for Social Sciences (SPSS) 20.0 package after it has been entered into the software package. Continuous data were presented as mean ± standard deviation and categorical variables were presented as frequencies and percentages.

Appropriate statistical methods were used, such as chi-square to compare the relationship between qualitative variable. Analysis of variance (ANOVA) was used to assess for significant associations between the means in quantitative variables. Correlation analysis was used to compare relationship between continous variables

III. RESULTS.

Two hundred and seventy five questionnaires were administered in all, fifty five participants for each class of obesity (Overweight, Stages 1,2,3) and 55 normal range BMI participants.All the questionnaires were completely filled and appropriate blood samples were collected.

DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS

The study is age and sex matched with 135 male and 140 female participants and the mean age for each sex categories being 43.73 and 43.54 respectively. The mean age as well as sex is also matched across the BMI stages with no statistical difference across the stages as shown in figure 4.2

Table 1.1: Distribution pattern of obesity among study participants based on gender**.**

BMI Stage	Male (%)	Female (%)	Total
Normal	28(51.9)	26(48.1)	54
Overweight	28(50.9)	27(49.1)	55
Stage one Obesity	26(47.3)	29(52.7)	55
Stage two Obesity	27(48.2)	29(51.8)	56
Stage three Obesity	26(47.3)	29(52.7)	55
Total	135(49.1)	140(50.9)	275

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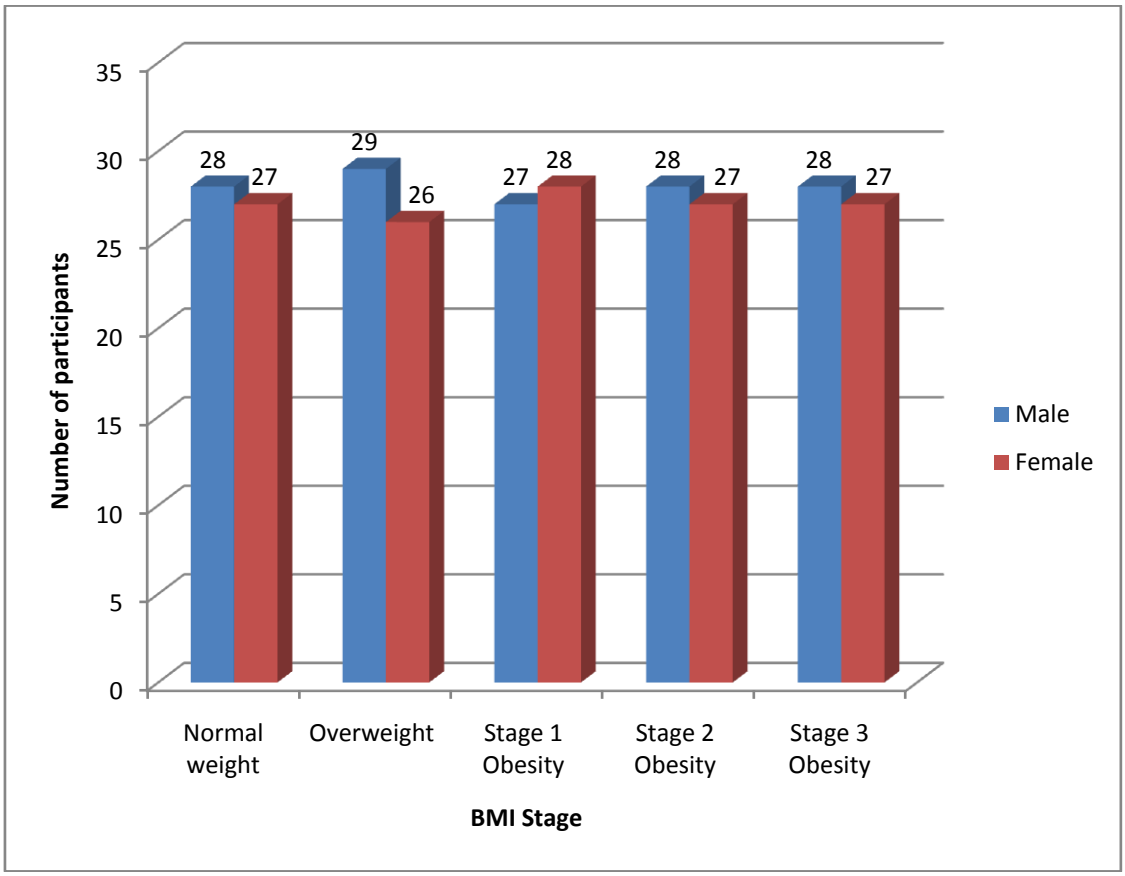

Figure 1.1: Bar Chart showing gender distribution among the participants and control.

ANTHROPOMETRIC MEASUREMENTS

There was a clinically significant progressive increase in the weight of study participants from control through the stages of overweight and obesity (59.70 ± 9.79, 73.60 ± 6.67; 86.18 ± 7.51; 92.45 ± 7.53; 105.58±6.10, p< 0.001) while the heights progressively decrease (1.65 ± 0.10, 1.64 ± 0.07, 1.64 ±0.07,1.58 ± 0.06,1.57± 0.04, p< 0.001). This gave a resultant clinically significant progressive increase in the mean BMI from the control through the various stages of overweight and obesity (21.92 ± 2.12, 27.31 ± 1.35, 32.16 ± 1.79, 36.98 ± 0.92, 42.87 ± 1.73 respectively p<0.001) as shown in table 4.2

Furthermore, while there was a statistically significant progressive increase in the mean hip circumference from control through the stages of overweight and obesity (93.56 ± 6.43, 105.47 ± 5.64, 114 ± 6.23, 122.32 ±3.63, 133.27 ± 16.33), the waist circumference increased only up to stage two obesity with a slight fall in stage three compared with stage two (78.65±8.33, 95.25 ± 6.28,103.25 ± 6.52,112.38 ± 8.69, 109.92 ± 6.54).

Post-hoc analysis however showed a clinically significant higher mean waist circumference in stage three than control, overweight and stage one obesity. This gave a resultant statistically significant progressive increase in the waist hip ratio from control through the stages of overweight and obesity (0.84 ± 0.06, 0.90 ± 0.06, 0.91 ± 0.06, 0.92 ± 0.01, 0.98 ± 0.10respectively p<0.001) as shown in Table 4.2,Although no statistical significance was seen in waist circumference between stage 2 and 3, also, no statistical significance in WHR between overweight and stage 2, stage 1 and stage 3(Table 4.3).

Sex categorization of all the participants showed a higher mean waist hip ratio in male participants than female participants across the stages of obesity and control while there was a lower BMI in male participants as shown in figure 4.3 and 4.4

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TABLE 1.2: ANOVA table showing anthropometrics measures of study participants.

Variables	C (N=54) Mean ± SD	OW (N=55) Mean ± SD	S1 (N= 55) Mean ± SD	S2 (N=56) Mean ± SD	S3 (N=55) Mean ± SD	p-value
Weight	59.70 ± 9.79	73.60 ± 6.67	86.18 ± 7.51	92.45 ± 7.53	105.58 ± 6.10	<0.001
Height	1.65 ± 0.10	1.64 ± 0.07	1.64 ± 0.07	1.58 ± 0.06	1.57 ± 0.04	<0.001
WC	78.65 ± 8.33	95.25 ± 6.28	103.25 ± 6.51	112.38 ± 8.69	109.92 ± 6.54	<0.001
HC	93.56 ± 6.43	105.47 ± 5.64	114.01 ± 6.23	122.32 ± 3.63	133.27 ± 16.33	<0.001
BMI	21.92 ± 2.12	27.31 ± 1.33	32.16 ± 1.79	36.98 ± 0.92	42.87 ± 1.73	<0.001
WHR	0.84 ± 0.06	0.90 ± 0.06	0.91 ± 0.06	0.92 ± 0.07	0.98 ± 0.10	<0.001

Key: C= control, OW= overweight, S1= stage one obesity, S2= Stage two obesity, S3= stage three obesity, **SD**= standard deviation, WC= waist circumference, HC= hip circumference, BMI= body mass index, WHR= waist:hip

CONVENTIONAL CARDIOVASCULAR RISK FACTORS MEASURED IN THE PARTICIPANTS AND CONTROLS

The mean systolic and diastolic blood pressures increase progressively from control to stage three obesity with both blood pressures exceeding the hypertension threshold only in stage three obesity, whereas post-hoc analysis showed no statistical significance when controls were compared with stage 1 and also controls with stage 3(Table 4.5)

The mean total cholesterol, triglyceride, low density lipoprotein cholesterol and fasting plasma glucose were lower in the controls and progressively increase across the stages of obesity of the participants, all being statistically significant with p value <0.001.Also post hoc analysis of total cholesterol showed no statistical significance when overweight was compared with stage 1,stage1 with stage ,low density lipoprotein cholesterol showed no statistical significance when control was compared with overweight, overweight with stage 1 also stage 1 with stage 2.Similarly triglyceride showed no statistical significance when control was compared with overweight, stage 2 with stage 3.(Table 4.5)

However, the high density lipoprotein cholesterol was highest in the controls and decreasing progressively across the stages of obesity of the participants, also at a statistically significant p value < 0.001.

TABLE 1.3: Clinical and laboratory parameters of study participants across various stages of obesity.

Variables	C (N=54) Mean ± SD	OW (N= 55) Mean ± SD	S1 (N= 55) Mean ± SD	S2 (N=56) Mean ± SD	S3 (N=55) Mean ± SD	p-value
SBP	118.67 ± 22.04	126.22 ± 10.45	129.77 ± 22.20	131.99 ± 21.53	148.61 ± 26.30	<0.001*
DBP	76.57 ± 10.94	85.75 ± 17.06	86.12 ± 7.41	88.52 ± 14.69	94.55 ± 17.0	<0.001*
TC	3.29 ± 0.67	3.89 ± 1.04	4.21 ± 1.06	4.60 ± 0.71	5.21 ± 1.03	<0.001*
HDL_c	1.07 ± 0.18	0.92 ± 0.30	0.78 ± 0.22	0.61 ± 0.09	0.59 ± 0.22	<0.001*

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LDL_c	2.01 ± 0.52	2.22 ± 0.30	2.53 ± 0.87	2.82 ± 1.06		3.73 ± 1.20	<0.001*
TG	0.91 ± 0.10	1.05 ± 0.50	1.14 ± 0.44	1.41 ± 0.56		1.48 ± 0.50	<0.001*
FPG	3.52 ± 0.61	3.90 ±0.87	4.29 ± 0.53	5.01 ± 0.62		5.55 ± 0.80	<0.001*

Key: C= control, OW= overweight, S1= stage one obesity, S2= Stage two obesity, S3= stage three obesity, **SD**= standard deviation, SBP= systolic blood pressure, DBP= diastolic blood pressure, TC= total cholesterol, HDL-c= High density lipoprotein, LDL-c = low density lipoprotein. TG= Triglyceride. FPG= fasting plasma glucose. * Statistical significance among various stages of Obesity and normal weight.

THE INFLAMMATORY MARKERS ACROSS THE STAGES OF OBESITY AND THE NORMAL BMI CONTROLS

The Serum hs-CRP in the controls was found to be low and falls to the low cardiovascular risk group (hs-crp value <1mg/L) while hs-crp levels increase across the stages of obesity in the participants, overweight participant fall into the intermediate cardiovascular risk group (hs-crp value between 1-3mg/L) and stages 1-3 participants fall into high cardiovascular risk group(hs-crp>3mg/L) and was statistically significant. This is as shown in table 4.6

The serum TNF-alpha in the controls was found to be within the normal reference range(TNF-a 1.2-15.3pg/ml) and increases across the stages of obesity above the reference range and was found to be statistically significant.

TABLE 1.4: Mean serum inflammatory markers across the stages of Obesity and normal weight

Variables	**C (N=54) Mean ± SD**	**OW (N= 55) Mean ± SD**	**S1 (N= 55) Mean ± SD**	**S2 (N=56) Mean ± SD**	**S3 (N=55) Mean ± SD**	**p-value**
Serum hs-CRP	0.93 ± 0.10	2.35 ± 0.44	3.27 ± 0.51	5.50 ± 0.74	6.97 ± 1.19	<0.001*
Serum TNF-a	6.57 ± 0.89	28.88 ± 4.67	33.36 ± 7.65	40.08 ± 1.04	51.92 ± 4.46	<0.001*

Key: C= control, OW= overweight, S1= stage one obesity, S2= Stage two obesity, S3= stage three obesity, **SD**= standard deviation, hs-CRP= highly sensitive C-reactive protein, TNF-a= tumour necrosis factor alpha. *Statistical significance among various stages of Obesity and normal weight.

CORRELATION OF INFLAMMATORY MARKERS WITH CONVENTIONAL CARDIOVASCULAR RISK FACTORS

There was a statistically significant correlation between the inflammatory markers (hs-CRP and TNF-a) and all the conventional cardiovascular risk factors measured in the study participants. While both systolic and diastolic blood pressures showed a weak but positive correlation, the total cholesterol, fasting plasma glucose and LDL cholesterol showed a strong but positive correlation.

However, the HDL cholesterol showed a strong but negative correlation with both inflammatory markers, hs-CRP and TNF-a with the correlation coefficients, r being -0.588 ($p< 0.001$) and -0.597 ($p< 0.001$) respectively.

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TABLE 1.5: Correlation between plasma hs-CRP, plasma TNF-alpha and conventional cardiovascular risk factors.

Variables (n= 275)	Plasma hs-CRP r (p-value)	Plasma TNF r (p-value)
SBP	0.198(0.0.001)*	0.203(0.001)*
DBP	0.228(0.001)*	0.257(<0.001) *
TChol	0.578(<0.001) *	0.557(<0.001) *
HDL-c	-0.588 (<0.001)*	-0.597 (<0.001)*
LDL-c	0.547(<0.001) *	0.482(<0.001) *
Trig	0.431(0.001) *	0.379(<0.001) *
FPG	0.697(<0.001) *	0.647(<0.001) *

Key: r= Pearson's correlation coefficient, SBP= systolic blood pressure, DBP= diastolic blood pressure, TC= total cholesterol, HDL-c= High density lipoprotein, LDL-c = low density lipoprotein. TG= Triglyceride. FPG= fasting plasma glucose. * Statistical significance among various stages of Obesity and normal weight. § Post hoc test with Bonferroni's correction

IV. DISCUSSION

The observations that raised concentrations of inflammatory markers especially hs-CRP in healthy subjects predicted the incidence of coronary heart disease (CHD) over a period of years suggested a role of inflammation in the initiation of atherosclerosis as well as in the precipitation of an acute event.[9] The synthesis of these inflammatory markers that are associated with cardiovascular disease especially coronary heart diseases have been linked with some traditional cardiovascular risk factors (Obesity, Hypertension, Diabetes Mellitus).The adipose tissue produces a variety of hormones and cytokines and thereby actively participates in a network of biomarkers that may be relevant for the development of CVD hence Obesity and overweight.[10]Researchers have found that plasma levels of CRP, TNF alpha and IL-6: markers of inflammation are elevated in subjects with obesity.[11]

The mean ages between the control and study groups in this study had no significant statistical difference and this allowed for comparison among the study participants. There was no significant statistical difference in gender (sex) participation in each of the study groups. The participants were age and sex matched.

The anthropometric indices measured weight, height, WC,HC while the BMI and WHR were calculated. The mean weight, BMI, WC,WHR of the study participants were higher than the controls which is statistically significant, this is expected since the study participants are the obese and overweight with increased deposition of adipose tissue.

The conventional cardiovascular risk factors measured in this study include Total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, fasting plasma glucose, systolic blood pressure and diastolic blood pressure. TC, LDLc, TG, FPG were significantly higher in the study participants when compared with the control group while HDLc is significantly lower in the study group when compared with the control group. S.Chaikate et al reported a similar increase in TC, FPG, TG and LDLc in obese individuals.[12] In obesity, adipose tissue T cells release cytokines contributing to development of insulin resistance[11] as depicted by increase in fasting plasma glucose. Chronic low grade systemic inflammation and insulin resistance in combination further leads to deranged metabolic profile.[11]Dyslipidaemia is a well known and major risk factor for ischemic heart disease as elevated levels of TG,TC, LDLc are documented risk factors for atherogenesis.[11] It has been shown that TC levels are continuously correlated with CHD risk over a broad range of cholesterol values in various populations throughout the world.[12]

Also, in this study we found elevated systolic blood pressure and diastolic blood pressure in the subjects when compared with the control group. The systolic and diastolic blood pressure shows an increased pattern through Overweight, Stage one, Stage two and Stage 3 obesity.

Obesity-related hypertension has grown substantially over this same time period, to the point where obesity is recognized as a major cause of high BP, and the combination of obesity and hypertension is recognized as a pre-eminent cause of CV risk.[13]

There is growing recognition that coronary heart disease (CHD) has an inflammatory component.[11]Hs-crp levels and TNF-α which are markers of inflammation, they were consistently found to be elevated over the increasing grades of

obesity. These inflammatory markers especially hs-crp and TNF-alpha have been reported to indicate the development of atherosclerotic linked CVD.

Pearson's correlation coefficient showed a significant association and positive correlation between the inflammatory markers measured in this study TNF-α and hs-crp with Triglycerides, LDL-cholesterol and Total Cholesterol levels as well as significant negative correlation with HDLc in the study group, thus indicating that with increasing body weight and worsening dyslipidemia which are traditional CVD risk, CRP and TNF-α values rise.

It is a well-known fact that obese individuals have a high incidence of development of coronary heart disease.[14] The pathogenesis of CHD associated with obesity has been linked with atherosclerosis. Inflammatory markers especially hs-crp and TNF-alpha have been reported to indicate the development of atherosclerotic linked CVD.[14]

The inflammatory markers were found to be elevated in the obese and overweight participants as compared with the normal BMI control participants and correlated significantly with traditional CVD risk factors: hypertension, Diabetes mellitus and Dyslipidemia.

V. CONCLUSION

The importance of this study was to determine the association of some inflammatory markerswith increasing body weight (overweight and obesity) and cardiovascular risk factors and to correlate these cardiovascular risk factors to the inflammatory markers.

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