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Unconventional Biomarkers: Early 'Snapshots' of Cardiovascular risk in Young Overweight and Obese adults

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ABSTRACT

Background:Atherosclerosis of the coronary arteries leads to a restriction in coronary blood flow. Multiple studies have demonstrated that total leukocyte, monocyte and neutrophil counts are significantly higher in CAD patients. Serum uric acidhas also been found to be an independent risk factor for both cardiovascular and renal diseases.Looking at the dearth of data regarding association of these risk markers in overweight and obese young adults, this study was carried out.Aim and Objectives: To study the correlation of pro-inflammatory markers of development of coronary artery disease in overweight and obese young adults.**Methodology**: A prospective cross-sectional study was conducted in a rural Tertiary Care centre for a period of two months.Two hundred medical students acted as study participants, and were subdivided into healthy controls, and an overweight/obese group. Anthropometric data, haematological, and biochemical parameters were obtained, and correlated with respective groups.Statistical Analysis:Chi square test and Student 't' Test were employed, with software used for the analysis being IBM SPSS Version 22.0, and p <0.05 being considered as significant.**Results**:Waist circumference and waist hip ratio (WHR) was significantly more in overweight and obese adults suggesting increased prevalence of visceral obesity. The monocyte percentage, neutrophil to lymphocyte ratio (NLR), and Serum uric acid levels were significantly elevated in overweight/obese category (p<0.05). **Conclusion**:The study demonstrated that overweight and obese young adults do have significantly increased levels of some unconventional biomarkers which can indicate future risk of cardiovascular diseases, and thus are indeed early "snapshots" which can be considered as warning signs. Early lifestyle modificationcan reduce the risk of cardiovascular disorders in such individuals.
Keywords: Coronary artery disease, Waist:Hip circumference, monocyte, Uric acid, lymphocyte, neutrophil.

Introduction

Coronary Artery Disease (CAD) is caused by atherosclerosis of the coronary arteries that leads to a restriction in the blood flow to the heart[1].It is the most common non communicable disease which is forecasted to be the major cause of morbidity and mortality in most developing nations by 2020[1]. A coronary event occurs somewhere in the world once approximately every 25 seconds, and approximately every 1 minute someone will die of one [2].

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Epidemiologic studies have identified high body-mass index as a risk factor for an expanding set of chronic diseases, including cardiovascular disease, diabetes mellitus, chronic kidney disease, many cancers, and an array of musculoskeletal disorders[2-4].Results from multiple studies of patients with and without CHD at baseline have indicated that the WBC count can be used to predict the incidence of coronary events. An increase in quartiles of WBC count from baseline over 24 hours after an MI episode has been correlated with an increase in all-cause death after a Myocardial Infarction (MI),5and a recent trials have shown that an increase in WBC count (defined as WBC count 6-24 hours after admission minus the WBC count on admision) was a significant predictor of death after PCI.6 Elevated levels of almost all subtypes of WBCs,

including eosinophils, monocytes, neutrophils, and lymphocytes (an inverse relationship), have been associated with increased risk of CHD.[7-12].Specifically, it has been demonstrated that total leukocyte, monocyte and neutrophil counts are significantly higher in CAD patients with low reactive hyperemia peripheral arterial tonometry index (RHI) (<0.57)compared with those with high RHI (≥0.57). Univariate analysis has also shown that RHI in CAD patients is positively correlated with total leukocyte, monocyte, and neutrophil counts.

Serum uric acid, a final enzymatic product of purine metabolism[13]. Although hyperuricemia has no universally accepted definition, it is generally defined as serum urate concentration in excess of 6.8 mg/dl.[14] In some studies, uric acid was found to be an independent risk factor for both cardiovascular and renal diseases[15]. Studies have demonstrated that the odds ratio for developing CHD was 2.59 for participants who had a serum uric acid level >9.0mg/dl[16] Studies in the paediatric age group have also found that hyperuricemia is an early marker of cardiovascular morbidity and the routine determination of circulating levels of sUA must be carried out for risk stratification in children. The effects of uric acid on the development of cardiovascular and renal diseases have been demonstrated in animal models and cell cultures[17]

There isa dearth of data regarding association of these risk markers in overweight and obese young adults, especially in rural areas. Thus, this study was carried out to evaluate these risk markers in young overweight/obese adults.

Aim:To study the correlation of pro-inflammatory markers with future risk of development of coronary artery disease in overweight and obese young adults.

Objectives:

•To estimate the prevalence of overweight and obesity in young adults.

•To assess the correlation of pro inflammatory markers Total Leucocyte Count (TLC), Differential Leucocyte Count (DLC), Serum Uric acid level, and Neutrophil: Lymphocyte ratio in the study subjects visa vice non obese normal healthy young adults.

•To correlate these inflammatory markers with various anthropometric parameters (waist circumference, waist: hip ratio).

Methodology

After obtaining due consent from the Institutional Ethics Committee, aprospective cross-sectional study with control group was conducted in the Dept. Of Medicine, and Central Clinical Laboratory, of a rural Tertiary care centre for a period of two months from 1st June 2018, to 31st July, 2018.

Study participants selected were 200 young adults(medical students)aged 18to 25 years, who, based on the ASEAN criteria applicable to Indian population, were subdivided into a healthy category, that acted as the control group, and overweight-obese.

Exclusion criteria considered was:

• Students having blood dyscrasias like Sickle cell trait.

• Students suffering from any infection or on anti-inflammatory medication, including

Glucocorticoid, or COX-inhibitor therapy, collagen vascular disorders.

Written consent of the participant was then obtained, having explained in detail about the purpose, methodology and implications of the study.

Waist circumference was taken next, in accordance with the WHO STEPS protocol, ensuring that the measurement was made at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest when the participant stood straight with two feet spread 25 to 30 cms apart, evenly distributing the body fat[13]

Following the protocolsmentioned in section 2.1.1 of WHO steps14, the hip circumference measurement was taken next, around the widest portion of the buttocks. Recommended values of <0.90, 0.90-1.0, and >1.0, and <0.80, 0.80-0.85, and >0.85 were considered as cut-offs for males and females respectively.

Next, BMI of participants was calculated as:

• Weight in kilograms recorded with the subject standing motionless on the standard weighing sale without foot wear, with light clothing using a portable weight scale and using inflexible bars with high accuracy.

• Height: Measured in cms to the nearest 0.5 cm with the subject standing in an erect position against a vertical scale without footwear an with the head positioned so that the top of the external auditory meatus will level the margin of bony orbit (Frankfurt's plane)

• Body Mass Index (Quetlet formula): BMI = Wight in kg / height in m^2

As has been identified with extensive research, Asian populations have different associations between BMI and percentages of body fat than their European peers. It has been concluded that the proportion of Asian people with a high risk of type 2 Diabetes and cardiovascular disease is substantial at BMIs lower than the existing WHO cut-off point for overweight (25 kg/m2).Thus, BMI values of the study, and control group will be calculated and classified intonormal BMI (18.5–22.9 kg/m2), overweight (23.0 –24.9 kg/m2) and

obese (≥25 kg/m2) based on the revised consensus guidelines for India.

Skin over the median cubital vein was then disinfected by applying surgical spirit over the cubital fossa, along with the application of a tourniquet proximal to the fossa, and using a sterile standard vermipuncture needle, blood was collected in a potassium ethylene diamine tetra acetate bulb for sampling and complete blood count, and analysed within 15 minutes of collection.Values of Total Leucocyte Count (TLC), Differential Leucocyte Count (DLC), and Neutrophil: Lymphocyte ratio was calculated using a ABX Pentra XL 80 CBC Analyzer manufactured by Horiba Ltd.

Statistical Analysis

Statistical Analysis were carried out using Inferential Statistics, including Chi square test and Student t Test. Software used for the analysis was SPSS Version 22.0, with p <0.05 being considered as significant.

Observation and results

The study assessed morphological, haematological and biochemical parameters of 200 candidates that were divided into categories identified as healthy and overweight/obese based on their BMI.Seventy young adults were found to have BMIs > 23 kg/m^2, and 130 of the remaining students had BMIs < 23 kg/m^2. Hence, 35% of the study population was found to be overweight/obese, while 65% served as healthy controls.

Weight of the students was the parameter considered next, and was found to correlate positively with students' BMIs. Mean Weight of the students was the parameter considered next, and was found to correlate positively with students' BMIs. Mean weight of healthy controls was 60.76 ± 8.90 kg, while overweight and obese students were found to have a mean weight of 74.19 ± 6.48 kg, with p value being <0.01, significant. Waist and hip circumferences of students was also found to correlate significantly with the category of BMI students were in. Healthy students had a mean waist and hip circumference of 78.75 ± 6.49 cm and 95.74 ± 8.66 cm respectively, while overweight and obese students had a mean waist and hip circumference of 90.41 ± 10.57 cm and 105.52 ± 9.58 cm respectively, p value being <0.01 for both, as depicted in Table 1.Waist:Hip ratio was also found to have a significant correlation, with the mean Waist:Hip ratio of healthy participants being0.82 ± 0.050, while the mean Waist:Hip ratio of overweight students came to be 0.87 ± 0.053, with p being <0.01.

Table 1: Comparison of Waist circumference, Hip circumference, and Waist/Hip ratio to BMI of students

S.no.	Parameter accessed	Category of students	Value:Mean ± SD	t-value and p-value
1.	Waist circumference	Normal	78.75 ± 6.49	79.09
		Overweight/obese	90.41 ± 10.57	p < 0.01
2.	Hip circumference	Normal	95.74 ± 8.66	53.76
		Overweight/obese	105.52 ± 9.58	p < 0.01
3.	Waist/Hip ratio	Normal	0.82 ± 0.050	18.21
		Overweight/obese	0.87 ± 0.053	p < 0.01

Table 2: Monocyte percentage in normal and overweight/obese category

BMI(kg/m^2)	N	Mean	Std. Deviation	Std. Error	Minimum	t-value and p-value
Normal	130	2.13	0.77	0.06	1.00	7.84
Overweight/obese	70	2.48	0.92	0.11	1.08	p=0.006,S
Total	200	2.25	0.84	0.05	1.00	

On comparison of individual parameters of Differential Leucocyte counts, only Monocyte percentages, being 1.94% ± 0.79 in normal healthy adults, and 2.24% ± 0.89 in overweight individuals, stood to be significant, with p value <0.01.

Table 3:Comparison of NLR in normal versus overweight/obese category

Sr. No.	Category of students	N	Mean	Std. Deviation	Std. Error	t-value and p-value
1.	Normal	130	1.94	0.790	0.069	6.02 p=0.015,S
2.	Overweight/obese	70	2.24	0.898	0.100	
3.	Total	200	2.05	0.839	0.056	

When Neutrophil/ Lymphocyte ratios (NLR) were compared, healthy participants had a mean ratio of 2.13 ± 0.77, while mean ratio in overweight /obese individuals was 2.48 ± 0.92. This proved to be significant with p value being 0.006

Table 4: Comparison of serum uric acid in normal versus overweight/obese category

Sr. No.	Category of students	No.	Mean	Std. Deviation	Std. Error	t and p-value
1.	Normal	130 (65%)	5.40	1.28	0.10	37.43 p=0.0001
2.	Overweight/obese	70 (35%)	6.50	1.15	0.14	
3.	Total	200 (100%)	5.79	1.32	0.09	

When serum uric acid levels were taken into consideration, healthy participants had a mean S. Uric acid level of 5.40 ± 1.28 mg/dl, while overweight individuals had a mean S. Uric acid level of 6.50 ± 1.15 mg/dl. This proved to be significant, with p value being <0.01. Multivariate Regression Analysis was carried out assuming S. uric Acid as the dependent variable. Correlation of S.Uric acid to other included parameters revealed that while there was no positive correlation between a participant's S. Uric acid levels and their gender, height, weight, waist circumference, hip circumference, Waist/Hip ratio, monocyte count, neutrophil count, lymphocyte count, eosinophil count, or basophil count, S. Uric acid did have a statistically significant association with Total Leucocyte Count and the Neutrophil/ Lymphocyte ratio of participants. Results of the Multivariate Regression Analysis are depicted in Table 5.

Table 5: Multivariate regression analysis assuming S. Uric acid as a dependent variable

Sr. No.	Parameters	Unstandardized Coefficients		Standardized Coefficients	t value	p-value
		B	Std. Error	Beta		
1.	Serum uric acid	10.304	27.466			
2.	Gender of students	-4.214	7.807	-0.215	0.540	0.590,NS
3.	Height of Students	0.116	0.099	0.919	1.164	0.246,NS
4.	Weight	-0.171	0.170	-1.348	1.002	0.318,NS
5.	Waist circumference	0.136	0.148	1.045	0.921	0.358,NS
6.	Hip circumference	-0.169	0.275	-0.394	0.612	0.541,NS
7.	Waist/Hip ratio	18.166	17.650	0.740	1.029	0.305,NS
8.	Total Leucocyte Count	0.000	0.000	0.182	2.358	**0.019,S**
9.	Monocyte count	-0.269	0.225	-0.170	1.197	0.233,NS
10.	Neutrophil count	-0.214	0.205	-1.186	1.046	0.297,NS
11.	Lymphocyte count	-0.140	0.209	-0.800	0.668	0.505,NS
12.	Eosinophil count	-0.119	0.208	-0.251	0.573	0.567,NS
13.	Basophil count	0.092	0.330	0.017	0.279	0.781,NS
14.	Neutrophil/ Lymphocyte ratio	0.965	0.465	0.617	2.078	**0.039,S**

Discussion

Obesity is a complex, multifactorial , chronic inflammatory disease[20], affecting, along with overweight, over a third of the world's population today[21,22]In recent years because of diets rich in fats, an increasing shift towards sedentary lifestyles, mechanized transport, and industrialization, prevalence of obesity has rapidly increased[23]. Obesity plays a central role in increasing the risk of cardiovascular disease[24].Although, obesity has been demonstrated to be a risk factor for atherosclerotic diseases, the mechanism of this increased cardiovascular risk in obese individuals has not been clarified[25]This study was carried out to assess the correlation of pro inflammatory markers in young obese adults and correlate these inflammatory markers with various anthropometric parameters. Our study demonstrated the positive correlation of BMI with TLC, Monocyte counts, serum uric acid and Neutrophil/Lymphocyte Ratio. Various epidemiological studies have shown a positive correlation between WBC count and risk of CVD both in healthy subjects free of CVD and in patients with CVD[26-28].In the EPIC-Norfolk Prospective Population Study, the higher risk for CVD associated with increased total WBC count seemed to be accounted for by the increased neutrophil count, but not lymphocyte or monocyte count[29].This was in contrast to findings we obtained, as monocyte counts was a parameter that was found to correlate significantly with BMI and future cardiovascular risk. Thus, a larger multi-centric study would be needed to confirm or refute the findings obtained.Neutrophils have been reported to migrate into ischemic myocardial tissue before other inflammatory cells and cause destructive changes by stimulating release of proteolytic enzymes, reactive oxygen radicals[30]. As a known fact, in the pathophysiology of atherosclerotic cardiovascular diseases, increased inflammation, and endothelial dysfunction play fundamental roles[31]. Outcomes of our study suggest that NLR may be one of the markers of inflammation in obese patients.There are some studies that suggest an independent association between the SUA level and C-reactive protein and other inflammatory markers (blood neutrophils, interleukin, TNF-α[32,33].In NHANES I, ARIC and Rotterdam studies, the high sUA level was associated with an increased risk of stroke[34-36].Similar to the results we obtained, the NHANES I study previously demonstrated a 48% increase in the risk of ischemic stroke in women for every 1.01 mg/dl increase in sUA. In ARIC study there was an independent and positive relationship between the incidence of the ischemic stroke and sUA [34]. However there are also some studies like ARIC study, Framingham study or an Austrian study, in contrast with our results did not found an independent association between the sUA levels and the increased risk of CAD[34,37]**.**Prevention of obesity and inflammation which start in early decades of life will contribute in decreasing rates of morbidity and mortality in adulthood. Although further studies are needed to confirm our results, the present finding underscore the notion that NLR and SUA can be called the early signs of cardiovascular diseases. Thus, even those who appear lean, or healthy or should ideally get routine screening done to check levels of sUA, DLC, and NLR, which can predict progression of the natural course of the disease.

Conclusion

Coronary Artery Disease, although being a disease primarily of the older population, can be prevented well in advance if necessary steps are taken from an early age, the most important being lifestyle modification. Treatment for CAD, aside from being prolonged, also often proves to be expensive for the patients. Thus, this study, by detecting adults at a higher risk of developing atherosclerosis in an early age using anthropometric, haematological, and biochemical parameters, aims to act as a source of primary prevention by education and advice on lifestyle modification.

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