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Research Article

Impact of waist circumference on red blood cells size in obese adults

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ABSTRACT

Background: Obesity is a chronic disease of multifactorial origin. Major cause for obesity is imbalance between energy intake and energy expenditure. One among the important public health problem in developing countries is increase in the prevalence of obesity. Obesity can be general obesity and central obesity. The central obesity is more dangerous than general obesity, since the central obesity reflects abdominal visceral fat, which acts as an endocrine organ and release several pro inflammatory cytokines, which leads to oxidative stress which in turn causes anisocytosis (variation in the size of the RBCs). Waist circumference indirectly measures this abdominal obesity. This study is aimed at finding the impact of Waist circumference on RBC size in obese adults.
Methods: This study was done in 100 subjects in the age group 25 to 50 years (50 control with normal waist circumference and 50 obese subjects with increased waist circumference) of Annapoorana Medical college hospitals. Patients with anaemia, malnutrition, and other chronic diseases who are RBCs may show anisocytosis were excluded. Waist circumference was measured. Peripheral smear was prepared and stained with leishman's stain. Smear was focused under oil immersion objective, and the image was captured using a digital camera. Image was transferred to the computer system and RBC diameter was measured using UTHSCA image tool software. Variation in size of the RBCs between control and subjects was compared using Pearson's product moment correlation coefficient and Paired sample t test.
Results: The variation in the size of RBCs (anisocytosis) was more in subjects with increased waist circumference when compared with subjects of normal waist circumference. By using Pearsons product moment correlation coefficient, significant r value of 0.5 and 0.7 were got in male subjects and female subjects respectively. Comparing waist circumference and anisocytosis using Paired sample t test, significant p value of < 0.05 was got in male and female obese subjects.
Conclusions: The oxidative stress caused by the cytokines released by the adipocytes causes decreased deformability of RBCs and cause anisocytosis, which further causes complications.

Keywords: Central obesity, Abdominal visceral fat, Waist circumference, Adipokines, Oxidative stress, Peripheral smear, Image tool, Anisocytosis

INTRODUCTION

Obesity has become a worldwide issue. Incidence and prevalence of obesity is increasing day by day because of change in life style patterns. We are living in an 'obesogenic environment', in which there is easy availability of energy-dense rich foods such as sweets, sugary drinks and fast food. Since the requirement for physical effort in home and at work is also reduced, there is continuous increase in the rate of over weight and obesity. Apart from the social stigma created, obesity acts as a key factor for diabetes, cardiovascular diseases, osteo arthritis, lipid disorders, sleep apnoea, and certain type of

cancers. Obesity has reached epidemic levels not only in developed nations but also in developing nations.[1] Obesity can be general obesity and central obesity. Indians have a genetic predisposition to central obesity, that arises from abnormal fat distribution in the body.[2] Having too much fat around the waist is more riskier than having fat in other parts of body. BMI (Body Mass Index) measures the general obesity and Waist circumference measures the central obesity.

Waist circumference provides an independent estimate of health risk beyond that provided by BMI because of its better reflection of visceral adiposity.[3,4] The measurement of abdominal visceral adipose tissue level has particular public health implication. With an increase in visceral adipose tissue, free fatty acids are easily directed to the liver for increased production of glucose, triglycerides and very low density lipoprotein.[5]

Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are used to measure abdominal visceral fat level. These methods require special and expensive equipments, and these are often unsuitable for screening of large groups of individuals in the community.

To screen abdominal visceral fat in large groups, waist circumference has been suggested as a desirable, simple, valid anthropometric indicator of visceral obesity since this is easily obtained and correlated with abdominal adipose tissue.[6] In obesity, there are few studies about the changes occurring in RBC membrane. Some of these changes lead to a higher rigidity and lower deformability of the RBC membrane.[7]

In this study we aimed at finding the impact of waist circumference on anisocytosis. Medical practitioners can explain the impact of waist circumference on RBC size and sensitise the obese patients attending their clinic about the deleterious effects of visceral adipose tissue.

METHODS

This study was conducted in 100 subjects between the age group 25-50 years who attended the Annapoorana Medical College Hospital. This study was started after getting ethical clearance from the ethical committee of Annapoorana Medical College and Hospitals. 50 (25 male and 25 female) subjects with normal waist circumference were included in the control group.

50 (25 male and 25 female) obese subjects with increased waist circumference were included in the test group. Written consent was got from all the subjects who participated in the study. Other parameters like lipid profile, blood pressure, total leucocyte count, haemoglobin were within normal limits in both the groups.

Modified tension tape was used for measuring the waist circumference. Fat tissue is a highly compressible tissue. So we get lot of inter measurer variations during waist measurement. In order to avoid this problem a pre calibrated spring balance was included into the normal measuring tape.

The calibration was done at 750gms using a standard weight suspension method. This helps to avoid inter measurer variability while taking the waist circumference measures. When the measurement was done, a tension of 750gm has to be applied which was the calibration mark in the spring balance. Waist circumference was measured in cms.

Ideal peripheral smears were prepared and stained with Leishman's staining. Smear was focused under oil immersion objective, and the image was captured using a camera. Image was transferred to the computer system and RBC diameter was measured using UTHSCA image tool software.

The calibration image was taken from the Neubauer's counting chamber smallest square of RBC which is 50 micron. Magnification factor was kept constant for all the images.

We compared the variation in the size of erythrocytes, between the control with normal waist circumference and subjects with increased waist circumference. Since the details of morphology of RBCs cannot be obtained by automated analysers, manual method of measuring anisocytosis was preferred in this study.

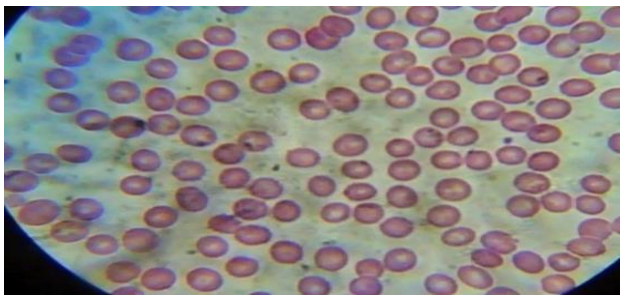

Image of peripheral smear from control, Largest -smallest RBC =7.9-6.2=1.7 microns.

Figure1: Image of peripheral smear from control.

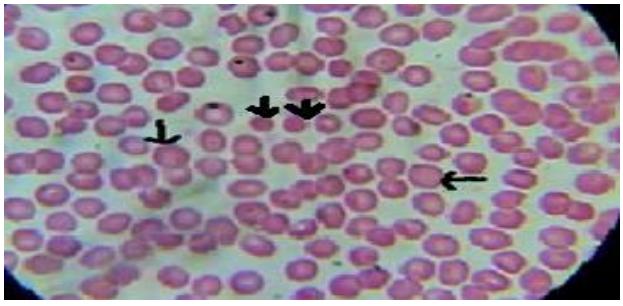

Image of peripheral smear from obese subject showing anisocytosis. Largest –smallest RBC= 9.6-5.9= 3.7 microns.

Figure 2: Image of peripheral smear from subject.

Best diagnostic support was given by Systematic examination of blood film and all the other test were either complimenting or confirming it.[8] Computer based image analysis method to determine red cell size, provides an accurate and reliable measurement, which is simple and cost effective.[9] The diameter of 25 RBCs were measured from each image. The variation in the size of RBCs (anisocytosis) (largest diameter_ smallest diameter), between the control and obese subjects were compared (Figure 1 and Figure 2).

RESULTS

We used Pearson's product moment correlation coefficient and Paired sample t test to find the correlation between waist circumference and anisocytosis. If the r value is near 1, there is significant correlation, if r value is positive there is positive correlation and if the r value is negative there is negative correlation.

Table 1: Correlation of waist circumfernce with anisocytosis using pearson's product moment correlation coefficient.

Variable	Male control r value	Male obese subject r value	Female control r value	Female obese subject r value
Waist circumference	0.2	0.5	0.1	0.7

The variation in the size of RBCs (anisocytosis) was more in subjects with increased waist circumference. Waist circumference was positively and significantly correlated with anisocytosis. The r values got after comparing the waist circumference and anisocytosis in male control and male obese subjects were 0.2 and 0.5 respectively. For female control and obese subjects the r values got were 0.1 and 0.7 respectively (Table 1). The normal variation in the RBC size is 1-2 microns.

Table 2: Variation in RBC size between control and obese subjects.

Study subjects	Range of RBC size (microns)
Male controls (n= 25)	6.2 to 7.9
Male subjects (n= 25)	5.8 to 10.2
Female controls (n= 25)	6.1 to 7.8
Female subjects (n= 25)	5.7 to 9.1.

Table 3: Comparison of waist circumference with anisocytosis using paired sample t –test.

Paired sample t –test			
Subjects.	Variable	Mean±SD	p value.
Control: female	Anisocytosis	1.7±0.2	
	Waist circumference	77±1.7	0.536
Male	Anisocytosis	1.7±0.2	
	Waist circumference	79±1.7	0.391
Obese female	Anisocytosis	3.3±0.1	
	Waist circumference	92± 3.3	0.000
Male	Anisocytosis	3.4± 0.4	
	Waist circumference	95±3.4	0.009

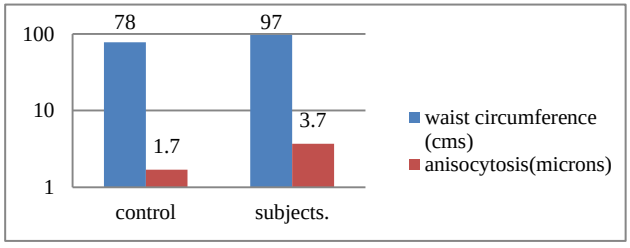

Figure 3: Correlation between waist cicumference and anisocytosis in male control and obese subjects.

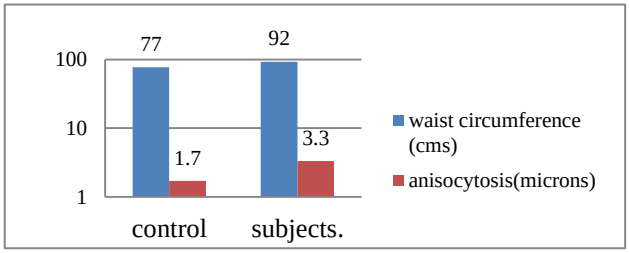

Figure 4: Correlation between waist circumference and anisocytosis in female control and obese subjects.

But, in obese subjects with increased waist circumference, the variation in the RBC size (anisocytosis) is 3.7 microns, which gives the significant r values. (Table2) By comparing the anisocytosis with waist circumference using Paired sample t-test, the significant p value of <0.05 was got in male and female obese subjects (Table 3) (Figure 3 and Figure 4).

DISCUSSION

From present study, we come to know that, waist circumference is positively and significantly correlated with anisocytosis in obese subjects. In that, the r value and p value were more significant in female obese subjects. Let us discuss about the results got in our study. Obesity is a chronic disease of multifactorial origin. Obesity can be defined as an increase in the accumulation of body fat. Waist circumference is a useful indicator of visceral fat distribution. Waist circumference is a better prognostic marker for obesity than BMI.[10]

Adipose tissue is not only an organ for storage of triglyceride, but it is also a producer of certain bioactive substances called adipokines, which are source of proinflammatory cytokines, such as TNF-α, IL-1, and IL-6. These cytokines are potent stimulators for the production of reactive oxygen species, which is responsible for increased oxidative stress (OS). Obesity, particularly central obesity, is an independent predictor of systemic oxidative stress.[11]

This is confirmed by increase in OS biomarkers, such as malondialdehyde (MDA) and F-2 isoprostanes (F2-IsoPs), in obese individuals, even in the absence of any chronic diseases such as diabetes, hypertension, hyperlipidemia etc in comparison to normal healthy individuals.[12] Two important mechanisms by which reactive oxygen species produces oxidative stress are 1) oxidation of fatty acids in mitochondria and peroxisomes, which produces ROS in oxidation reactions, 2) over-consumption of oxygen, which generates free radicals in the mitochondrial respiratory chain.

Because of the increase of adipose tissue, the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), are also significantly diminished. Superoxide dismutase protects RBCs from the harmful effects of oxidative stress. Glutathione peroxidase catalyzes the breakdown of inorganic and organic peroxides and prevents lipid peroxidation and protects the cell membrane from oxidative damage.[13]

The red blood cell (RBC), is a non-nucleated cell. So it exhibits a very limited biosynthesis capacity and poor repair mechanisms. So they are vulnerable to physical and/or chemical stress. Oxidative stress causes oxidative damage to to the lipids and proteins in the RBC membrane. The erythrocyte is a good model to study the oxidative damage of lipids and proteins occurring in pro inflammatory and oxidative conditions.[14] Higher oxidative stress leads to reduced erythrocyte survival and results in anisocytosis due to an increase in the proportion of circulating premature erythrocytes.[15] So we compared waist circumference and anisocytosis in this study. Anisocytosis is caused by free radicals by the following mechanisms:- a) Deoxidation of RBC membrane lipids which alters the structural integrity of the membrane; b) Peroxidation of the cell membrane lipids changes the membrane permeability.[16]

In general, the overall effect of lipid peroxidation is to decrease membrane fluidity, deformability, visco elasticity.[17] Oxidative stress also causes, conformation changes in membrane cytoskeleton protein which alters fluidity of the membrane, erythrocyte shape, size and osmotic fragility.[18]

The level of nervonic acid (a fatty acid normally present only in sphingomyelins- rigidifying agent of the cell membrane) was significantly higher in the obese group.[19] In the study done by Giovanna, they found out by spin label method that the fluidity of the RBC membrane was decreased in obese patients even in the absence of remarkable changes in plasma lipids and lipoproteins.[20]

The reason for the significant r and p values got in female subjects is, a higher peroxidation level is seen in females compared to males, which is due to the higher percentage of fat possessed by females.[21] In the study done by Hermsdorff et al, they have found that that urinary F2-isoprostanes is more in women than in men, which leads to unfavorable redox state in young women compared to men.[22]

CONCLUSION

More than 500 million people worldwide are currently affected by obesity both in developed and developing countries.[23] It is the most common nutritional disorder in humans from wealthy societies.[24]

Apart from the social stigma caused by obesity, the obese population is prone for diabetes, hypertension, cardiovascular disorders, arthritis, sleep apnoea, endocrine disorders etc. Currently obesity is a serious public health problem. In our study we found positive correlation between waist circumference and anisocytosis.

Adipocytes release pro inflammatory cytokines, which stimulates oxidative stress, which increases the reactive oxygen species, which increases the rigidity of the RBC membrane and decreases the deformability of RBC membrane leading to anisocytosis.

This decrease in erythrocyte membrane fluidity in obese patients reduces the rate of blood flow (in particular in the microcirculation) and the oxygen diffusion through the erythrocyte membrane and its exchange with tissues,

which causes the further complications.[25] Obesity shows its deleterious effect of increased oxidative stress on every other cells in the body even in the absence of other risk factors and hence should be treated. Waist circumference can be used as a reliable tool to measure abdominal fat and this can be used to sensitise the obese patients in the community.

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