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THE STUDY OF BIOMARKERS LEPTIN AND ADIPONECTIN TO ASSESS THE CARDIOVASCULAR RISK IN OBESE PATIENTS UNDERGOING BARIATRIC SURGERY

[1]HARSHAL NAIK, [2] DR. GEETA IBRAHIM, [3] DR. SANJAY BORUDE

[1] Researcher, Institute of Science Department of Biochemistry, Mumbai
[2] Principle, Nirmala Niketan, College of Home Science, Churchgate, Mumbai
[3] Bariatric Surgeon, Bariatrics by Borude's 21, Prabhuta Villa, Kemps Corner, Mumbai
E-mail: [1] harshalnaik89@gmail.com, [2] 2teekay7@rediffmail.com, [3] bariatricbyborudes@gmail.com

Abstract

Due to changes in lifestyle, dietary habits and reduction in physical activity owing to urbanization, modernization and westernization, obesity seems to increase at an alarming rate right from childhood. Modernization has brought along with it the fast-food culture, high intake of fatty food consumed due to the taste factor that comes along with it, increasing levels of stress, depression, etc. leading to a large population being prone to obesity with a high risk of developing cardiovascular diseases. Therefore, people prefer opting for Bariatric Surgery in order to lose weight. The biomarkers- Leptin, Adiponectin, Small Dense LDL and Leukocyte telomere length have been studied to predict the risk of cardiovascular diseases. It would also help in providing scope for future research in the overall management if risk factors associated with cardiovascular diseases.

I INTRODUCTION

Cardiovascular diseases (CVD) encompass any medical conditions related to the heart and blood vessels. CVD is the main cause of disability and premature death worldwide, and is projected to remain the leading cause of death. Persons with high levels of cholesterol in blood, a condition called as hypercholesterolemia, are more prone to atherosclerosis. It is not a disease but a metabolic derangement that can be caused by many diseases, notably cardiovascular disease. "Hyperlipidemia" (elevated levels of lipids in the blood) and "hyperlipoproteinemia" (elevated levels of lipoproteins in the blood) are the other factors responsible for CVD. Elevated cholesterol in the blood is due to abnormalities in the levels of lipoproteins, the particles that carry cholesterol in the bloodstream. Cholesterol contains various subtypes that include very-low-density lipoprotein cholesterol (VLDL-C), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). The normal cholesterol range in human body should be below 200 mg/ dl and is good for body to function normally and anything over 240 mg/ dl indicates risk for developing CVD. Relative risk of all lipoproteins in CVD has been extensively studied and till now LDL-C, known as bad cholesterol, is considered as a marker for cardiovascular risk assessment. The LDL-C level in human body should be below 130 mg/dl. High density lipoprotein cholesterol, which is known as good cholesterol and have antioxidant property, should range between 35-40 mg/dL. The high level of HDL-C (~60 mg/dL) has the benefit of reducing the chances of heart attack. Moreover, there are several amendable risk factors that include hyperlipidemia, dyslipidemia and have higher incidence of deranged lipid profile. The various study established that elevated LDL-C and increased ratio of LDL/HDL (high density lipoprotein) is the most important lipid derangement making those subjects more susceptible to atherosclerosis.

II OBESITY AND CARDIOVASCULAR DISEASE

Obesity is recognized as a classic risk factor for atherosclerosis and subsequent cardiovascular disease. It is becoming a global epidemic in both children and adults. It's a known fact that obesity can take a toll on our heart health. And there's a plethora of research to back it up. For instance, a recent study published in April 2018 in the journal JAMA Cardiology concluded that adults between

ages 40 and 59 who are overweight or obese have a significantly increased risk (ranging from 21 to 85 percent higher) of developing cardiovascular disease as compared with their normal weight peers. Individuals who are overweight, defined as having a body mass index (BMI) between 25 and 29.9, or are obese (a BMI of 30 or higher), also have a much greater risk of developing cardiovascular disease at a younger age. Obesity is associated with an increased risk of morbidity and mortality as well as reduced life expectancy. The research showed that individuals who are obese had a shorter lifespan. In fact, obesity is an independent risk factor for CVD, and CVD risks have also been documented in obese children. Reducing cardiovascular (CV) disease (CVD) risk is a primary public health imperative given the substantial morbidity and mortality associated with the disease. The most effective nonpharmacologic means of achieving such risk reduction is weight loss. Even a moderate 5% to 10% weight loss through diet and lifestyle interventions has been shown to decrease the risk for conversion from impaired glucose tolerance to overt diabetes and can maintain blood pressure reductions over prolonged periods of follow-up. More dramatic weight loss after bariatric surgery has been associated with even greater benefits, such as reduced CV mortality and total mortality in obese patients, particularly those with diabetes.

Health service use and medical costs associated with obesity and related diseases have risen dramatically and are expected to continue to rise. Besides an altered metabolic profile, a variety of adaptations/alterations in cardiac structure and function occur in the individual as adipose tissue accumulates in excess amounts, even in the absence of comorbidities. Hence, obesity may affect the heart through its influence on known risk factors such as dyslipidemia, hypertension, glucose intolerance, inflammatory markers, obstructive sleep apnea/hypoventilation, and the prothrombotic state, in addition to as-yet-unrecognized mechanisms. On the whole, overweight and obesity predispose to or are associated with numerous cardiac complications such as coronary heart disease, heart failure, and sudden death because of their impact on the cardiovascular system. However, the cardiovascular clinical evaluation of obese patients may be limited because of the morphology of the individual. In this statement, we review the available evidence of the impact of obesity on CVD with emphasis on the evaluation of cardiac structure and function in obese patients and the effect of weight loss on the cardiovascular system.

Overweight and obesity is a complex disease itself and is linked to more deaths worldwide than underweight. Obesity is now recognized as the first leading cause of premature mortality followed by cancer and DM, and the biggest issue behind this incidence is the association between obesity and CVD. Obesity has long been considered as an established risk factor for CVD. For instance, in a Framingham cohort study, relative weight (i.e., percentage of desirable weight) was found to be positively and independently associated with a 26-year incidence of CAD, stroke, HF, and CVD-related death. Recent studies have further investigated how both the duration and degree of obesity affect the risk of different CVDs. Data from the Coronary Artery Risk Development in Young Adults study showed that, for every 2 years lived with obesity, the risk of CVD mortality significantly increased by 7%. As for the risk of different types of CVD, another meta-analysis revealed that the relative risk (RR) of stroke with obesity was 1.3, and that of VTE with obesity was 2.4. The risk of HF increased 5% for men and 7% for women per 1 unit increase in BMI (27–30).

THE SOCIOECONOMIC BURDEN OF OBESITY AND IMPACT ON THE OVERALL HEALTH

Being overweight or obese refers to the condition that a person's weight is higher than what is considered as a healthy weight for a given height. BMI has been conventionally used as an indicator to define overweight and obesity. This is a person's weight in kilograms divided by the square of height in meters. For adults, Centers for Disease Control and Prevention and World Health Organization (WHO) define BMI of 18.5 to 25 as the normal range, 25 to 30 as overweight, and 30 or higher as obesity, while age also needs to be taken into consideration for children. Although BMI is not a perfect index to assess the relationship between the body weight and health of an individual for its incapability of diagnosing the body fatness, it is still considered the most useful screening tool at an individual level worldwide.

Overweight and obesity are growing global issues, in terms of prevalence, health risks, and socioeconomic impact. Globally, more than 1.9 billion adults aged 18 years and older, nearly 40% of the world's population, were overweight in 2016. Over 650 million people—approximately 13% of the adult population— were obese. The worldwide prevalence of obesity nearly tripled between 1975 and 2016. If this rate is kept constant, almost half of the world's adult population will be overweight or obese by 2030.

As another important part of the obesity epidemiology, the prevalence of obesity in a country does not directly correlate with its economic status. When the countries were categorized according to the income level as low-income, lower middle income, higher middle-income, and high-income, the prevalence of obesity increased up to upper middle-income countries, however the high-income countries came to the second among the 4 categories (20). Low socioeconomic status of individuals has been associated with a higher prevalence of obesity regardless of the nation's economic status, whether it is developing, transitional, or developed. Larger disparities in individual access to better quality diet (e.g., fresh fruits, vegetables, and fish) were observed especially in countries with developing or transitional economies as they faced the globalization of food markets. This is likely due to greater economic inequality, under-established healthcare systems, and poorer education on diet. Development of strategies to address overweight- and obesity-related health problems is therefore warranted worldwide regardless of the nation's economic status.

Besides physical health problems, the economic impact of obesity has been an important public health issues. Obesity imposes large socioeconomic costs not only to the healthcare system but also to the society. Recent studies on the association between BMI and costs attributable to obesity have described that the burden comes in the form of the individual's lost productivity as a result of lost work days, lower productivity at work, mortality, and permanent disability. All of these could lead to a loss of economic growth nationwide. Above all, medical costs for obesity-related diseases have been the biggest global concern. Medical costs are typically divided into direct costs and indirect costs. Direct costs include costs for the treatment

and management of the diseases—e.g., inpatient and outpatient care. An example of direct non-medical costs is transportation to healthcare providers. Indirect costs include early mortality costs and morbidity costs due to sickness absence and informal care costs. Costs of obesity have been calculated in several studies. In the US, the direct per-capita costs over a lifetime amounted to US$171,482 in 2010, and the total 10-year per-capita costs were predicted to be US $70,200 in 2013 (33). Costs of obesity on the individual, families, and nations have been more enormous than ever, calling not only for global healthcare policy reforms but also for better treatment and preventative interventions on individual basis.

IV SMALL DENSE LDL: A NEW MARKER

Sd LDL cholesterol is a subtype of LDL cholesterol, one of the lipoproteins. Lipoproteins transport lipids in the blood stream and are characterized depending on size and weight. LDLs vary in size through genetic determination and dietary lipid intake. They range from small dense through normal size to big buoyant LDL. They all transport triglycerides and cholesterol to the tissues, but their atherogenesis varies according to size. Smaller particles such as sdLDL more readily permeate the inner arterial wall and they are also more susceptible to oxidation.

Modern research on CVD decodes several new risk factors that include elevated lipoprotein(a), high sensitivity C reactive protein (hs- CRP), fibrinogen and hyperhomocysteinemia etc., an important one of which is estimation of small dense LDL cholesterol (sd LDL-C). Lipoprotein profiles that are relatively rich in sd-LDL particles are associated with up to a 3-fold greater risk of myocardial infarction than those mainly consist of large buoyant (lb)-LDL particles. Currently published report established that predominance of sd-LDL-C is a major component of an atherogenic lipoprotein phenotype, and a source of increased risk for coronary heart disease. The ratio of sd-LDL-C to LDL-C plays an important role in assessing CVD.

V ADIPONECTIN:

A protein hormone produced and secreted exclusively by adipocytes that regulates the metabolism of lipids and glucose. Adiponectin influences the body's response to insulin. Adiponectin also has anti-inflammatory effects on the cells lining the walls of blood vessels.

High blood levels of adiponectin are associated with a reduced risk of heart attack.

Low levels of adiponectin are associated are found in people who are obese (and who are at increased risk of heart attack).

VI LEPTIN:

A hormone produced mainly by adipocytes (fat cells) that is involved in the regulation of body fat. Leptin interacts with areas of the brain that controls hunger and behavior and signals that the body has had enough to eat. A small number of people have genetic mutations in the leptin gene, leading to a greater demand for food, resulting in obesity.

Obesity is a risk factor for cardiovascular diseases. Leptin levels are increased in obesity and leptin exhibits cardiovascular actions that may contribute to increased cardiovascular risk.

In patients with acute myocardial infarction, obesity is related to increased leptin. The subcutaneous fat compartment seems to be an important determinant of plasma leptin, concentration. Leptinemia is associated with several biochemical disorders suggesting that leptin may be pathogenetic factor in cardiovascular disease.

VII ADIPONECTIN/LEPTIN RATIO

Adiponectin/Leptin ratio is used as an estimator of dysfunctional adipose tissue. Obesity is characterized by an increase in circulating leptin concentrations, along with a decrease in the levels of adiponectin in the blood. This reflects the obesity associated alterations in adipose tissue adipokinome. The adiponectin/leptin ratio has been suggested as a marker of adipose tissue dysfunction. This ratio decreases with increasing number of cardiometabolic risk factors reflecting the functionality of adipose tissue. With the expansion of adipose tissue, the expression and secretion of adiponectin decreases, which produces a drop in circulating concentrations. In parallel, expression and secretions of leptin increases which triggers a boost in the blood levels. Thus, as BMI increases with obesity, the adiponectin/leptin ratio decreases. Since the adiponectin/leptin ratio reflects the functionality of adipose tissue, this ratio may be clinically useful to identify subjects susceptible to cardiometabolic diseases. According to previous data, we considered that an Adpn/Lep ratio equal or higher to 1.0 (with adiponectin concentrations expressed in µg/mL and leptin levels in ng/mL) can be considered normal, a ratio between 0.5 and 1.0 can indicate moderate-medium increased risk, and a ratio below 0.5 suggests a severe increase in cardiometabolic risk.

VIII BARIATRIC SURGERY:

Traditional treatments to achieve weight loss such as diet, lifestyle, and behavioral therapy have proven relatively ineffective in treating obesity and associated cardiovascular risk factors in the long term, especially when used in isolation, but have demonstrated some metabolic and cardiovascular benefits when they are used together as combination strategies. It is important to note that these treatments have been specifically ineffective on the morbidly obese subgroup of patients (BMI _40 kg/m2) and have led to development of operations in the form of "bariatric surgery" to treat obesity and its comorbidities.

Bariatric surgery is a type of surgery performed on morbidly obese individuals to achieve weight loss. Weight loss is achieved by reducing the size of the stomach with a gastric band or through removal of a portion of the stomach (sleeve gastrectomy or biliopancreatic diversion with duodenal switch) or by resecting and re-routing the small intestine to a small stomach pouch (gastric bypass surgery). The U.S. National Institutes of Health recommends bariatric surgery for obese people with a body mass index (BMI) of at least 40, and for people with BMI of at least 35 and serious coexisting medical conditions. The selection of the type of bariatric operation performed depends on surgical and patient preference. These procedures can be classified into 3 categories: restrictive malabsorptive, or combination

procedures. Restrictive operations literally decrease the size of the stomach (either by a synthetic gastric band, stapling, or size reduction by "sleeve gastrectomy"), leading to satiety with smaller volumes of food that eventually leads to food intolerance and weight loss. Malabsorptive operations consist of bypassing segments of bowel, which thereby cause malabsorption of nutrients (such as the biliopancreatic diversion with or without duodenal switch and ileal interposition). The combination group of operations involves both aspects of restriction and malabsorption such as the Roux-en-Y gastric bypass, which is considered as the "gold standard" bariatric operation and is currently the most commonly performed procedure for weight loss worldwide. These bariatric operations demonstrate the most encouraging results for rapid weight loss and subsequent improvements in overall morbidity and life expectancy in obese patients. Long-term follow-up of bariatric patients reveals significant reductions in mortality from heart disease, diabetes mellitus, and cancer. This leads to a decrease of any-cause mortality by 40% while also cutting long-term healthcare costs. Consequently, these operations have found a role in decreasing cardiovascular risk in asymptomatic obese patients but can also reduce cardiac mortality and morbidity in obese patients with established cardiac pathology.

IX METHODOLOGY:

1. Around 20-25 obese patients in age group 16-65 years males & female have been recruited. These patients have been admitted in various hospitals in Mumbai under bariatric surgeon Dr. Sanjay Borude.

2. An informed consent is obtained from all subjects to be participants in the study. Ethical clearance is obtained from the Ethics Committee of Breach Candy Hospital for carrying out the study.

3. Blood samples of the patients was collected and Leukocyte telomere length would be estimated using PCR, small dense LDL using ELISA kit, Leptin using Human leptin ELISA kit and Adiponectin using Human Adiponectin ELISA kit.

4. All participants were questioned about previous and current diseases, use of medications. MI waist circumference & waist to hip ratio were measured & calculated.

5. Measurement of Telomere Length (Δ value) by qPCR

   Genomic DNA is extracted from blood samples by standard procedures of chloroform extraction. Purified DNA samples is diluted in a TE buffer (PH7.5) It is dissolved by heating at 95 °C for 5min.in dry bath & quick chilled by transfer to an ice/water bath for 5 min.& stored at 4 °C until the time of assy. The purity & concentration of extracted DNA is checked on spectrophotometer by taking ratio of 260/280.This DNA is used for checking the expression of telomere gene. (A quantitative PCR method for measuring telomere length)

X. RESULTS:

The analysis for Leptin, Adiponectin and Small Dense LDL has been carried out using ELISA Kits.

A high proportion of the subjects included in the study were classified as obese, while other individuals were considered lean according to BMI. According to the Adpn/Lep ratio, 10 subjects had a ratio equal or higher to 1.0, 13 individuals exhibited a ratio equal or higher to 0.5 and lower than 1.0, and 51 subjects presented a ratio lower than 0.5. BMI was significantly higher ($p < 0.001$) in the ≥0.5–<1.0 and <0.5 groups as compared to the ≥1.0 group. The Adpn/Lep ratio was significantly lower in individuals with obesity (Lean: 2.00 ± 1.98; Obese: 0.26 ± 0.19; $p < 0.001$).

XI. CONCLUSION:

The main finding of the study is that the Adpn/Lep ratio is a reliable biomarker to predict the cardiovascular risk in obese patients. Obesity and CVD are characterized by increased levels of leptin and decreased levels of adiponectin. This results in the reduction of adiponectin/leptin ratio which is indicative of a dysfunctional adipose tissue. Since the Adpn/Lep ratio reflects the dysfunction of adipose tissue, we and others have postulated that this ratio may be clinically useful to identify subjects susceptible to cardiometabolic diseases.

Traditional biomarkers like the Lipid Profile Test are used for routine screening, however, they underestimate the cardiovascular risks. These novel biomarkers are stronger and stable predictors which would provide more accurate results and therefore, this study would be immensely helpful in evaluation of risk in young age group which would help detection, followed by treatment, modification of lifestyle, which would ultimately decrease the onset of CVD.

The Adpn/Lep ratio, and the proposed cut-off points, may be an interesting and useful estimator of obesity- and associated cardiometabolic risk, allowing the identification of a higher number of subjects at risk.

Adpn/Lep Ratio	**≥1.0**	**≥ 0.5 <1.0**	**<0.5**	***p***
n	10	13	51	
Age	48 ± 17	54 ± 16 *	48 ± 14 †	0.038
Weight, kg	64 ± 9	77 ± 16 *	91 ± 13 *, †	<0.001
BMI, kg/m2	21.7 ± 2.5	26.9 ± 5.3 *	39.8 ± 7.9 *, †	<0.001
Leptin, ng/mL	5.2 ± 2.9	10.4 ± 6.9	40.9 ± 26.6 *, †	<0.001
Adiponectin, µg/mL	12.2 ± 7.3	7.4 ± 4.0 *	6.8 ± 3.5 *	<0.001
Adpn/Lep ratio	2.83 ± 2.16	0.74 ± 0.15 *	0.21 ± 0.11 *, †	<0.001

Therapeutic strategies can be aimed at increasing the adiponectin/leptin ratio. The first approach for accomplishing this aim is weight loss. It is well known that dietary restriction and weight loss increase adiponectin levels and reduce leptin concentrations, which render an increase in the adiponectin/leptin ratio. The increased adiponectin/leptin ratio induced by weight loss eventually leads to reduced atherosclerosis risk in obese patients.

Bariatric surgery remains the most effective option for achieving important and sustained weight loss. It has rapidly expanded, due to its capacity to induce sustained long-term weight loss, ameliorate obesity-related comorbidities, and reduce mortality, constituting an effective option for individuals with severe obesity. Further prospective studies

will assess the involvement of the Adpn/Lep ratio after bariatric surgery in the prevention of serious obesity-related complication.

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