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Original Article

Biochemistry Section

Fibrinogen Levels in Obese and Normal Individuals

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ABSTRACT

Introduction: Obesity is a risk factor for Cardiovascular Disorders (CVDs). Adipose tissues associated with obesity secrete large number of peptide hormones, cytokines and non-peptide biologically active molecules. Interleukin-6 (IL-6) is a key regulator that modulates acute phase fibrinogen synthesis and high levels of fibrinogen leads to increased platelet aggregation and thrombus formation, which ultimately lead to coronary artery disease.

Aim: To evaluate the fibrinogen levels, and correlate them with IL-6 levels in obese and normal weight individuals.

Materials and Methods: The present cross-sectional study was conducted in SRM Medical College Hospital and Research Centre, Chennai, Tamil Nadu, India. Sixty obese individuals aged 20 to 65 years, and 60 age and sex-matched normal weight individuals attending the Master Health Check-Up and Medicine OP was selected for the study. Serum IL-6 was measured by Enzyme-linked Immune Sorbent Assay (ELISA) and fibrinogen was measured by antigenic method. Statistical analysis was done using student's t-test and Pearson correlation analysis. The p-value <0.05 was considered as statistically significant.

Results: The mean fibrinogen level in obese individuals (407.74±38.31 mg/dL) was significantly higher (p-value <0.001) than the normal weight healthy controls (287.49±52.46 mg/dL). The mean IL-6 level in obese individuals (78.52±8.95 pq/mL) was also significant (p-value <0.001) higher than the normal weight healthy controls (9.41±6.15 pq/mL). Statistically significant correlation (r-value 0.521 and p-value <0.001) was observed between serum IL-6 and fibrinogen level in obese individuals.

Conclusion: Fibrinogen plays a major role in platelet aggregation in the final step of coagulation cascade. It is a major determinant of plasma viscosity, erythrocyte aggregation and formation of fibrin. Increased level of fibrinogen seen in obesity is associated with increased risk of Coronary Artery Disease (CAD) and myocardial infarction.

Keywords: Adipose tissue, Coronary artery disease, Interleukin-6, Myocardial infarction, Obesity

INTRODUCTION

Overweight and obesity are the most prevalent lifestyle related health problems. Excessive weight and obesity is a risk factor for the development of chronic diseases such as cardiac, respiratory disease, non-insulin dependent diabetes mellitus or Type 2 Diabetes Mellitus (T2DM), hypertension and cancers. Obesity is defined as a condition of abnormal or excessive fat accumulation in the adipose tissue of the body due to an energy imbalance which leads to an increased storage of energy, mainly as fat. Currently, more than 1 billion adults worldwide are overweight and at least 300 million of them are clinically obese [1]. As per 2014, World Health Organisation (WHO), Global Health Observatory (GHO), United States (US) has the highest prevalence rate of overweight and obesity (62% for overweight in both sexes, and 26% for obesity), while South East Asia (14% overweight in both sexes and 3% for obesity) has the lowest prevalence rate [2]. India has the third-highest number of obese and overweight people (11% of adolescents, and 20% of all adults) after US and China. [3].

Adipose tissue has an important role in obesity. Adipose tissue can respond dramatically to alterations in excess nutrient intake through adipocytes hypertrophy and hyperplasia [4]. Adipose tissue secretes large number of peptide hormones, cytokines (IL-6) and non-peptide biologically active molecules such as activated lipids [5]. Interleukin-6 (IL-6) is a key regulator, which modulates acute phase fibrinogen synthesis [6]. Fibrinogen plays an important role in pathophysiological processes in the body, including atherogenesis and thrombogenesis [7-9]. Infiltration of fibrinogen into the vessel wall results in increased platelet aggregation, increase in blood viscosity and thrombus formation and ultimately leads to CAD [10].

Based on these findings, the study was designed to evaluate IL-6 and fibrinogen levels in obese and normal weight individuals.

MATERIALS AND METHODS

This cross-sectional study was conducted from January 2016 to May 2016 at SRM Medical College Hospital and Research Centre, Chennai, Tamil Nadu, India on individuals attending the Master Health Check-Up Programme and Medicine Outpatient. The study protocol was approved by the Institutional Ethical Committee (ECN: 140/ICE/2016). The sample size was calculated using the statistical formula n=4 pq/L^2, and 60 obese individuals (aged 20-65 years) and 60 age and sex-matched normal weight healthy individuals (controls) were selected as per Indian Council of Medical Research (ICMR) guidelines. Any individual with Body Mass Index (BMI) >23 is termed overweight, and BMI >25 as obese [11].

Individuals with acute/chronic infection or on treatment for diabetes, hypertension, thyroid, CAD, arthritis and rheumatoid arthritis were excluded from the study. The purpose of the study was explained to all the participants and written informed consent was obtained before commencement of the study.

The demographic details, relevant history and anthropometric measurement were recorded. Total Body fat percentage and subcutaneous fat percentage was measured using OMRON HBF 375 body composition monitor. Overnight fasting blood sample (5 mL) was collected in sodium citrate and plain vacutainer under aseptic precaution by skilled phlebotomist. For quantification of serum IL-6, a portion of blood samples were allowed to clot at room temperature for 30 minutes, and then centrifuged at 1000 x g for 10 minutes. The sera obtained were analysed using ELISA.

Plasma fibrinogen levels were measured using KC 4 Delta four channel coagulation analyser (Indian BioSystem).

STATISTICAL ANALYSIS

Data was analysed using Statistical package for scientific studies (SPSS) version 21. The results were represented as mean±Standard deviation (SD). Student's t-test was used to analyse the difference between the mean levels of various parameters. Correlation between various variables was assessed using Pearson's correlation equation. The p-value <0.05 was considered statistically significant.

RESULTS

Plasma fibrinogen and serum IL-6 levels were measured in both obese and in normal weight individuals. Mean fibrinogen and serum IL-6 levels were significantly (p-value <0.001) higher in obese when compared to the normal weight individuals [Table/Fig-1].

Parameters	Obese (n=60)	Normal (n=60)
Age	35±7	42±9
Height (m^2)	155.43±8.00*	165.65±6.83
Weight (kg)	79.36±7.94*	58.41±5.15
BMI (kg/m^2)	32.27±2.70*	21.03±1.17
WC (cm)	104.10±10.35*	70.93±4.23
HC (cm)	110.35±6.38*	90.80±4.16
WC-HC ratio	3.24±14.56	0.78±0.05
Total body fat %	35.42±4.56*	25.47±3.76
Subcutaneous fat (%)	30.05±4.09*	23.76±3.46
Fibrinogen level (mg/dL)	407.74±38.31*	287.49±52.46
IL-6 level (pq/mL)	78.52±8.95*	9.41±6.15

[Table/Fig-1]: Anthropometric measurements and biochemical parameters of obese and normal individuals.
BMI: Body mass index; WC: Waist circumference; HC: Hip circumference; WC-HC ratio: Waist hip ratio. Values expressed as Mean±SD. Students t-test. *p-value <0.001 statistically significant

Gender-wise analysis showed that both fibrinogen and serum IL-6 levels were significantly (p-value <0.001) higher in obese men/women as when compared to the normal weight men/women. Within obese group, men had higher fibrinogen and serum IL-6 levels as compared to women [Table/Fig-2].

Parameters		Obese	Normal
Sex	Female	20	24
	Male	40	36
Fibrinogen (mg/dL)	Female	400.8±37.8*	278.24±46.52
	Male	419.5±36.1*	299.74±47.18
IL-6 (pq/mL)	Female	74.70±7.2*	5.16±3.97
	Male	82.78±9.7*	10.72±4.82

[Table/Fig-2]: Biochemical parameters of obese and normal individuals based on gender.
Values expressed as Mean±SD. Students t-test. *p-value <0.001 statistically significant

Positive correlation (p-value <0.001) was observed between IL-6 and BMI (r-value=0.446), WC (r-value=0.697), HC (r-value=0.718), fibrinogen (r-value=0.521) and between fibrinogen and BMI (r-value=0.722), WC (r-value=0.848), HC (r-value=0.877) [Table/Fig-3].

Parameters		BMI (kg/m^2)	WC (cm)	HC (cm)	WC-HC ratio	IL-6 (pq/mL)	Fibrinogen (mg/dL)
IL-6 (pq/mL)	r-value	0.446*	0.697*	0.718*	0.087	1	0.521**
	p-values	<0.001	<0.001	<0.001	0.508		<0.001
Fibrinogen (mg/dL)	r-value	0.722*	0.848*	0.877*	0.136	0.521**	1
	p-values	<0.001	<0.001	<0.001	0.300	<0.001	

[Table/Fig-3]: Pearson's co-relation analysis between various biochemical parameters (BMI, WC, and WC/HC ratio), serum IL-6, and fibrinogen in obese individuals (n=60).
IL-6: Interleukin-6; BMI: Body mass index; WC: Waist circumference; HC: Hip circumference; WC-HC ratio: Waist hip ratio. Correlation is significant at the 0.05* and highly significant at the 0.01** level (2-tailed)

DISCUSSION

Obesity is a global epidemic and a risk factor for diseases like atherosclerosis, CAD, diabetes mellitus, breast cancer and colonic cancer [3]. Obesity also plays a cardinal role in the inflammatory response [12]. The present results demonstrate significance differences in serum Inflammatory marker (IL-6) levels between the obese and control groups. Bastard JP et al., reported positive correlation between circulating IL-6 and the degree of obesity estimated by BMI, waist circumference and WC/HC [13]. The possible known mechanism whereby serum IL-6 levels increased in obesity is due to the fact that obese individuals have relatively more adipose tissues. Adipose tissue is known to produce signalling pathway regulating local and synthetic pro-inflammatory response, especially the release of cytokine IL-6 [14,15]. However, the signalling pathways involved in adipocyte IL-6 release are not completely known. Orban Z et al., study suggest the role of β3 Adrenergic receptors (ADRB3) in the trigger expression of pro inflammatory genes including IL-6, MCP-1, PAI-1. This ADRB3 mediated inflammation depends on activation of Hormone Sensitive Lipase (HSL) suggesting involvement of lipolytic products as pro-inflammatory mediators and triggering the inflammatory response especially through sphingosine kinase. [16]. Mohamed-Ali V et al., reported that in the obese subjects secretion of IL-6 by adipose tissue was 25% higher in the basal state [12]. Kern PA et al., also reported that BMI have a positive co-relation with cytokine level (IL-6), are secreted by adipose tissue and IL-6 secretion was 30% higher in obese individuals [17]. Similar findings were reported by various other studies [18,19].

Literature suggests that fibrinogen have a strong linear relationship with traditional risk factors like obesity. Stec JJ et al., reported strong linear relationship between fibrinogen and obesity, estimated by BMI [10]. Herwig H et al., also reported significant association between fibrinogen and degree of obesity estimated by BMI, WC, HP and waist:hip ratio [20]. In the present study significantly higher fibrinogen level were observed in obese individuals as compared to normal individuals. In addition, some studies reported that fibrinogen levels are associated with obesity and cardiovascular events, such as CAD [21,22]. Similar findings were reported by Nagel G et al., in overweight and obese children [23]. Another study among adolescents and children also demonstrated a close relationship between fibrinogen, obesity and physical fitness, which was implicated in cardiovascular morbidity and mortality later in life [24].

In the current study, high positive correlation was observed between fibrinogen levels and BMI/obesity, and the inflammatory response marker (release of IL-6). A 7-fold increase in IL-6 levels and fibrinogen level in circulation was reported by Roytblat L et al., [15]. Fibrinogen is a key component of the inflammation and clotting pathways and an established risk factor for CVD. Fibrinogen production is unregulated in response to cytokines released during inflammation. Fibrinogen constitutes of two sets of α, β, and γ-chains that are coded by three genes namely *FGA*, *FGB* and *FGG* present on human chromosome 4. Although each gene is separately transcribed and translated, transcription is well coordinated, and increase in expression of one gene, up-regulates the expression of other genes [25]. IL-6 is the key regulator, though other mediators, such as glucocorticoids, IL-1β and TNF-α also modulate acute phase fibrinogen synthesis [6]. Acting via an intracellular pathway, IL-6 binds to its receptor and activates STAT proteins. In particular, STAT3, also known as acute phase response factor, is the major transcription factor mediating regulation of IL-6-responsive genes [25]. STAT3 relays signals from the IL-6 receptor to the nucleus where it binds to response elements in the promoter regions of the fibrinogen genes. Human fibrinogen genes are highly responsive to the IL-6 cytokine, and leads to the production of fibrinogen [6].

LIMITATION

Small sample size and more in depth work on the role of inflammatory markers are needed to evaluate its association with fibrinogen and obesity for the prevention of future risk of CHD.

CONCLUSION

The present study concludes that there is a significant co-relation between plasma fibrinogen and serum IL-6 concentrations in the degree of obesity. The results highlight the potential of inflammatory pathways as targets for CVD prevention, and need for studies of IL-6 signalling inhibition for the prevention of coronary artery disease.

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