DOI: 10.7860/JCDR/2022/51602.16119

Original Article

Biochemistry Section

Estimation of Proinflammatory Cytokines and Mediator CD40 Ligand Levels in Young Tribal Subjects of Tripura- An Observational Study

DEBAPRASAD CHAKRABARTI[1], PARTHA SARATHI PAL[2]

ABSTRACT

Introduction: Obesity is the established risk factor for the development of cardiovascular diseases like hypertension, diabetes mellitus, chronic heart diseases and atrial fibrillation. Cytokines such as Interleukin-6 and Interleukin-12 (IL-6, IL-12) play a significant role in the development of obesity related disorders. Cluster of differentiation 40 (CD40) ligand, a trimetric protein structurally related to Tumour Necrosis Factor alpha (TNF-α) family contributes to atherothrombosis by being a link between platelets, inflammation, thrombosis and atherogenesis. The present study was undertaken to explore the interplay between inflammatory cytokines and CD40 Ligand (CD40L) in young obese tribal subjects.

Aim: To estimate the level of proinflammatory cytokines like IL-6, IL-12 and CD40 ligand in obese subjects and compare with non obese tribal subjects of Tripura, India.

Materials and Methods: In this observational study, 60 non obese and 60 young obese tribal subjects within age group of 18-36 years were enrolled, over a period of two years from March 2014 to March 2016 at Tripura Medical College (TMC) and Dr. BRAM Teaching Hospital, Tripura, India. Serum levels of IL-6, IL-12 and C-reactive Protein (CRP) were estimated by Enzyme-Linked Immunoassay (ELISA). Plasma level of fibrinogen activity was measured by coagulation assay. The Homeostasis Model Assessment (HOMA) of insulin resistance was calculated as insulin (micro unit per milliliter) x glucose (milimoles/Ltr)/22.5. Soluble Cluster of differentiation 40 Ligand (sCD40L) in serum was determined by ELISA. Statistical analysis was performed using Mann-Whitney test, Chi-square test and regression analysis.

Results: Cohort consisted of 19 (31.1%) males and 41 (68.3%) females whereas the control non obese group comprised of 16 (26.6%) males and 44 (73.3%) females. In the present study, the authors had observed that the biochemical parameters like fasting blood glucose, cholesterol, triglycerides and High-Density Lipoprotein (HDL) cholesterol were significantly higher in obese subjects compared to non obese subjects. Further, the authors observed that CRP, IL-6, IL-12 and fibrinogen level were statistically higher in obese subjects. A statistical significant difference was found in fasting insulin level and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) between obese and the non obese group. Finally, there was positive relationship between CD40 ligand and cytokines with obesity.

Conclusion: The present study has demonstrated that the circulating levels of IL-6 and IL-12 have a strong relationship with obesity and other parameters of metabolic risk. It was also observed that there is a clear positive relationship between CD-40L and cytokine families.

Keywords: Adipose tissue, Interleukins, Metabolic risk, Obesity

INTRODUCTION

Obesity is a complex disorder and a major health risk factor linked to diabetes, hypertension, stroke, Cardiovascular Disease (CVD), cancer as well as early death [1]. Central obesity is closely associated with insulin resistance, lipid disorders, oxidative stress and low grade chronic inflammation resulting in accelerated atherothrombotic process. The levels of several acute phase response proteins and proinflammatory cytokines are significantly increased in both atherosclerosis and obesity [2,3].

Cytokines such as IL-6 originating from adipose tissue have a fundamental role in the pathogenesis of CVD. IL-6 acts synergistically with other interleukins and growth factor and enhances circulating platelet count [4]. Similarly IL-12, another proinflammatory cytokine has received increasing attention in the development of obesity related disorders [5].

Insulin resistance is the central pathophysiologic cause of metabolic syndrome. Insulin resistance which is assessed by HOMA-IR is related with several cardiovascular risk factors like hyperglycaemia, dyslipidaemia, obesity and inflammation [6]. HOMA-IR has been proved to be a robust method for the surrogate assessment of insulin resistance [7].

Fibrinogen has a strong impact on blood coagulation and platelet aggregation. It has also been shown to have direct influence on the vascular wall as well as play the role of acute phase reactant. These features provide the pathophysiological mechanistic link between fibrinogen and CVDs. Furthermore, an increased level of fibrinogen is positively correlated with higher risk of developing CVD [8,9].

The CD40L system has been implicated in the pathogenesis of atherothrombotic complications in CVDs and as well as in inflammation and thrombosis [10].

The CD40 ligand and its soluble counterpart sCD40L is a trimetric protein structurally related to TNF alfa super-family. It binds to CD40, on cells such as endothelial cells, monocytes and dendritic cells [11,12]. Normally absent from the surface of unstimulated platelets, CD40L is rapidly presented to the surface after platelet activation [13]. Platelet associated CD40L on exposure to CD40 expressing cells induces the expression of adhesion molecules, the release of inflammatory cytokines and procoagulant tissue factor [14].

The data on the extent of proinflammatory and prothrombotic cytokines is mainly derived from western population and remains largely unknown in obese indigenous tribes of India. Whereas an accepted convention of wisdom exists for Indian tribes that they are suffering from under nutrition and away from lifestyle related diseases. However, studies have shown, Indian tribal population are experiencing phenomenal changes on socio-cultural and economic

front because of the rapid urbanisation, lifestyle changes and dietary habits leading to increasing prevalence of obesity [15,16].

A study has shown that not only there is a trend of increasing prevalence of obesity in tribal population of India; they are at higher risk than the general Indian population [17]. With this background, the present study has been designed to explore the correlation between inflammatory cytokines and CD40L among young obese and non obese subjects which is not well studied in North Eastern part of India previously.

MATERIALS AND METHODS

This was an observational study, conducted at Tripura Medical College and Dr. BRAM Teaching Hospital, Tripura, India, after due approval by the Institutional Ethical Committee {approval no.F.3 (PO-75) Ethical/SFTMC/2010-11/4015, Date 08-10-2013}. The study period was two years from March 2014 to March 2016. All the subjects were enrolled after obtaining written informed consent.

Inclusion criteria: During the study period, total of 60 subjects belonging to ethnic population with central obesity attending Medicine Outpatient Department of TMC and Dr. BRAM Teaching Hospital served as cases in the study. For comparison, equal numbers of non obese healthy tribal subjects (attendant of the patients) were included randomly which served as controls in the study. The study population was between 18-36 years.

Exclusion criteria: Subjects suffering from atherosclerotic diseases and having impaired renal or liver function and connective tissue disorders, cancers and those taking steroid treatment were excluded from the study. Neither the obese subjects nor the non obese had any active infectious or inflammatory disease.

Sample size calculation: Going through the hospital census annually on an average 30-40 young adult patients of tribal ethnicity attended at Medicine OPD of TMC and Dr. BRAM Teaching Hospital over the three years preceding the study period. As only a limited number of patients within the prespecified age group were seen in outpatient setting, all the 60 tribal subjects who attended Medicine OPD between March 2014 to March 2016 were included in this study.

Samples for obese tribal subjects were collected using census sampling technique and for non obese tribal subjects were taken according to convenient sampling technique.

Study Procedure

All the participants underwent complete medical examination, Blood Pressure (BP) and anthropometric measurements. The anthropometric variables like weight, height, Body Mass Index (BMI) and waist circumference were verified in duplicate considering the mean value of two measurements. According to the National Health, Lung and Blood Institute (NHLBI), BMI is calculated as weight in kilograms divided by the square of height in meters (kg/m^2) [18]. According to the WHO recommendation, BMI cut-off points are <16 kg/m^2 (severe underweight), 16.0-16.9 kg/m^2 (moderate underweight), 17-18.4 kg/m^2 (mild underweight), 18.5-24.9 kg/m^2 (normal range), ≥25 kg/m^2 (overweight), 25-29.9 kg/m^2 (preobese), ≥30 kg/m^2 (obesity) [19]. Waist circumference was measured using a stretch resistant tape that provides a constant 100 gm tension placing it at the midpoint between the lower margin of least palpable rib and the top of iliac crest. The WHO recommended cut-off for Waist Hip ratio (WHR) ≥0.90 for men and ≥0.85 for women considered as central obesity [20].

Resting BP was measured in sitting position after 15 minutes of rest using a mercury manometer and a cuff appropriately sized for arm size of the subject. Blood samples were taken after overnight fasting and collected into pyrogen free tubes at room temperature. Collection tubes were then centrifuged at 3000 rpm for 20 minutes and serum samples were stored at -20°C in numerous aliquots until use. Fasting Blood glucose (GOD-POD method), Serum Cholesterol (CHOD-PAP method), Triglyceride (GPO-Triender method) and HDL (Direct) were measured in automated equipment (Erbachem 5 plus V2, Erba Mannheim) following manufacturer's (Erba) instructions.

Serum CRP, IL-6 and IL-12 were assayed with commercial enzyme linked immunosorbent assay in Lisaquant-TS (Tulip Diagnostic) ELISA reader. Each assay was performed according to the manufacturer's protocol. All biochemical measurements were performed at the same time in order to avoid procedural variations. The cut-off value of CRP (Abnova), IL-6 (Diaclone SAS) and IL-12 (Diaclone SAS) were taken upto 10 mg/L, <2 pg/mL and 2.2 mg/mL respectively by ELISA in Lisaquant-TS ELISA reader [21-23].

Fasting blood insulin and fibrinogen were measured by ELISA in Lisaquant-TS ELISA reader and coagulometric methods in Sysmex coagulation analyser respectively. The cut-off value of Insulin (Diagnostic automation) was taken between 2-25 µIU/mL and for fibrinogen (Diagone Ltd.,), it was from 2-4g/L [24,25]. The estimate of insulin resistance was determined by means of HOMA- IR as follows: fasting blood insulin concentration (mU/L) X fasting glucose concentration (mmol/L) divided by 22.5. The cut-off value of HOMA-IR was taken 2.5 [26].

Soluble CD40L was estimated by ELISA in Lisaquant-TS ELISA reader immediately or after one to three freeze thaw cycles and at different centrifugation speeds as described by Schoenbeck U et al., [27]. The cut-off value of sCD40L (Biovision Incorporated) is <6 pg/mL.

STATISTICAL ANALYSIS

The statistical analysis was performed with Statistical Package for Social Science (SPSS) (version and 13.0, Chicago IL). Normality of the data was checked by Shapiro-Wilk test. The unpaired Mann-Whitney U test was used to assess statistical difference between two independent groups. In correlation analysis, normally, distributed data was analysed with Pearson's correlation whereas Spearman's correlation was used for non normal data. Multivariate linear regression analysis was performed to assess the independent relationships between sCD40L levels and other variables after adjusting for confounding factors. Results were presented as Mean+SEM. The p-value <0.05 was considered significant.

RESULTS

The study included 60 obese subjects and 60 non obese control subjects with mean age of 30.47±3.75 years and 27.20±6.89 years, respectively. Male and female ratio of study subjects and controls were 1:2.1 and 1:2.7 respectively. Anthropometric, clinical and laboratory characteristics of the obese and non obese population are described in [Table/Fig-1].

In this study, the authors observed that parameters like fasting blood glucose, serum cholesterol, serum triglyceride and HDL cholesterol were significantly higher in obese cases compared to non obese subjects.

Similarly the plasma levels of CRP, an inflammatory marker, IL-6 and IL-12 the proinflammatory cytokines, fibrinogen a prothrombotic factor and CD40L (index of platelet activation) were significantly higher in obese subjects compared to non obese. Statistically significant differences were also found in fasting insulin level and HOMA-IR between obese and the non obese group [Table/Fig-1].

The present study showed that a clear correlation exists between elevated IL-6 with rising level of blood glucose, IL-12, fibrinogen, and sCD40L in obese group compared to the non obese population [Table/Fig-2].

Statistically significant association was also observed between IL-12 and levels of triglyceride, CRP, IL-6, fibrinogen, and sCD40L in obese subjects only [Table/Fig-3].

Study also demonstrated that an elevated concentration of sCD40 L was associated with rising levels of CRP, IL-6, IL-12, fibrinogen, insulin and HOMA-IR in the obese group, and it was statistically significant [Table/Fig-4].

Variables		Obese (n=60)	Non obese (n=60)	p-value
Age	≤30 year	37 (48.1%)	40 (51.9%)	0.568$
	>30 year	23 (53.5%)	20 (46.5%)	
Gender	Male	19	16	0.68$
	Female	41	44	
BMI (kg/m^2) Mean (±SD)		28.9140 (±0.95)	21.1723 (±1.34)	<0.001*
Waist: Hip ratio		0.95 (±0.06)	0.92 (±0.09)	0.015*
Glucose (mg/dL)		155.90 (±56.31)	109.62 (±23.41)	<0.001*
Serum cholesterol (mg/dL)		194.55 (±29.40)	176.17 (±23.13)	<0.001*
Serum triglyceride (mg/dL)		204.45 (±65.99)	176.17 (±23.13)	<0.001*
HDL (mg/dL)		37.52 (±4.72)	35.45 (±2.37)	0.031*
CRP (mg/dL)		4.12 (±2.25)	2.59 (±1.25)	0.001*
IL-6 (pg/mL)		2.48 (±1.05)	1.02 (±0.47)	<0.001*
IL-12 (pg/mL)		2.78 (±1.62)	1.40 (±1.50)	<0.001*
Fibrinogen (g/L)		3.78 (±2.29)	1.75 (±0.14)	<0.001*
CD40 L (pg/mL)		25.74 (±14.62)	5.58 (±1.54)	<0.001*
Insulin (µIU/mL)		10.67 (±7.88)	6.08 (±6.22)	<0.001*
HOMA-IR		6.22 (± 6.15)	2.85 (±3.81)	<0.001*
Platelet (lac/cumL)		235483.33 (±61492.87)	238683.33 (±51672.53)	0.529*

[Table/Fig-1]: Anthropometric, clinical and laboratory characteristics of the obese and non obese population.
$p-value calculated by Chi-square test, *p-value calculated by Mann-Whitney U test; *p-value <0.05 is statistically significant

Variables	IL-6			
	Obese (n=60)		Non obese (n=60)	
	Correlation value	p-value	Correlation value	p-value
Obesity	0.504	<0.001*	0.109	0.406
Waist: Hip ratio	0.039	0.77	0.112	0.395
Fasting blood glucose	0.298	0.021*	-0.145	0.270
Serum cholesterol	-0.034	0.797	0.131	0.318
Serum triglyceride	0.064	0.627	0.085	0.520
HDL	0.116	0.375	-0.108	0.410
CRP	0.405	0.061	0.072	0.583
IL-12	0.452	<0.001*	0.180	0.169
Fibrinogen	0.551	<0.001*	-0.001	0.994
CD40 ligand	0.518	<0.001*	0.237	0.068
Fasting blood insulin	0.228	0.079	-0.230	0.077
HOMA-IR	0.170	0.194	-0.282	0.029
Platelet	-0.100	0.445	-0.180	0.893

[Table/Fig-2]: Association between IL-6 with other inflammatory cytokines and other variables.
*p-value <0.05 is statistically significant; When data normally distributed Pearson correlation was used and for non normal data spearman rank correlation was used

Variables	IL-12			
	Obese (n=60)		Non obese(n=60)	
	Correlation value	p-value	Correlation value	p-value
Obesity	0.698	<0.001*	0.137	0.295
Waist:Hip ratio	0.029	0.825	-0.071	0.591
Glucose	0.236	0.070	-0.107	0.417
Serum cholesterol	-0.020	0.882	-0.010	0.938
Triglyceride	0.260	0.045*	-0.055	0.676
HDL	0.143	0.276	-0.011	0.931
CRP	0.613	<0.001*	0.035	0.793
IL6	0.452	<0.001*	0.180	0.169
Fibrinogen	0.697	<0.001*	0.082	0.531
CD40L	0.310	0.016*	-0.083	0.526
Insulin	0.175	0.181	-0.178	0.173
HOMA-IR	0.107	0.414	-0.094	0.475
Platelet	-0.101	0.441	-0.032	0.811

[Table/Fig-3]: Association between IL-12 with other inflammatory cytokines and other variable in cases.
*p-value <0.05 is statistical significant; When data was normally distributed Pearson correlation was used and for non normal data spearman rank correlation was used

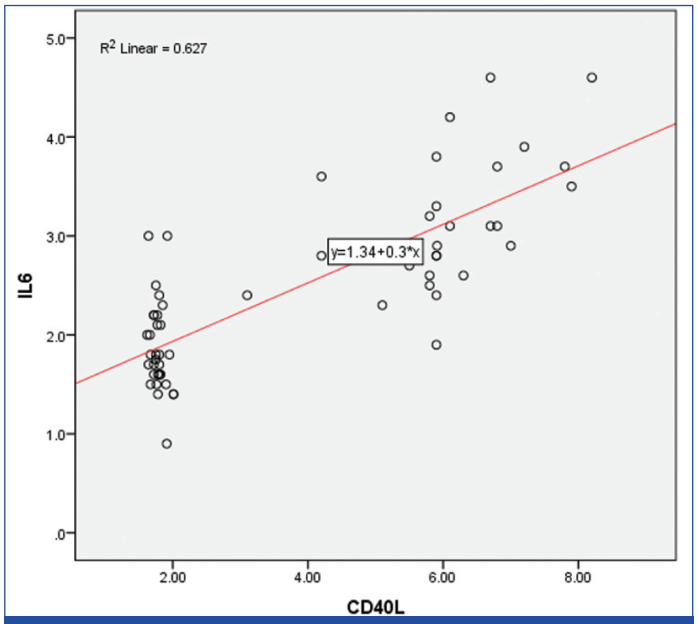
Variables	sCD40L			
	Obese (n=60)		Non obese (n=60)	
	Correlation value	p-value	Correlation value	p-value
IL-6	0.518	<0.001*	0.221	0.090
IL-12	0.310	0.016*	-0.201	0.124
Fibrinogen	0.394	0.002*	-0.318	0.013*
Insulin	0.270	0.037*	-0.260	0.045
HOMA- IR	0.281	0.029*	-0.453	<0.001*
CRP	0.268	0.039*	0.014	0.915
Platelet	-0.075	0.567	9.02	0.877

[Table/Fig-4]: Correlation between CD40L with different markers in obese.
*p-value <0.05 is statistically significant
When data normally distributed Pearson correlation was used and for non normal data spearman rank correlation was used

After applying the multivariate logistic regression analysis, it was observed that only the value of IL-6 remained significant and independent predictor high sCD40L level (p=0.001) [Table/Fig-5,6].

Independent variables	sCD40L		
	Adjusted R^2	Std. coefficients beta	p-value
CRP	0.230	0.009	0.945
IL-6		3.408	0.001*
IL-12		0.793	0.431
HOMA- IR		0.577	0.566

[Table/Fig-5]: Multiple linear regression analysis.
*p-value=0.001 is statistically highly significant



[Table/Fig-6]: Scatter diagram showing relationship between IL-6 and sCD40 ligand.

DISCUSSION

It was observed that proinflammatory cytokines like IL-6, IL-12 and CD40 Ligand are significantly elevated in young obese tribal population compared to non obese cohorts. As such rising trend of obesity is seen in the young Indian tribes, it is a matter of great concern for their future cardiovascular health and other obesity related diseases [17]. While burden of this cardiometabolic risk factor

should be viewed in a regional and ethnic context; data regarding obesity in indigenous population is sparse.

In the present study, most individuals in the obese cohort had multiple cardiometabolic risk factors. It was observed that high levels of CRP, an inflammatory marker was present in more than one quarter of study population.

It was observed that IL-6, present substantially in adipose tissue, was significantly higher in obese subjects compared to non obese subjects. This pleotrophic cytokine exhibited a significant increase in parallel with obesity and showed positive correlation with fasting blood sugar, fibrinogen and sCD40 Ligand. Similar observation was made by Roytblat L et al., in their study on obese adolescent population [28]. Likewise El-Mikkawy DME et al., in their study concluded that high circulating level of IL-6 signals the intensity of chronic and systemic inflammation that results with high grades of obesity and this might contribute to the development of atherosclerotic vascular diseases both directly and indirectly through alteration of HDL cholesterol [29]. Similar observation was also made by Baikpour M et al., who showed that a significant positive correlation exist between serum level of IL-6 and obesity with BMI ≥25 kg/m^2 [30].

It is well recognised that IL-6 cytokine has multiple effects ranging from inflammation to host defense, tissue injury and modulating insulin resistance. It has been seen that high levels of IL-6 are predictive of type 2 diabetes and myocardial infarction in adults and ablation of this proinflammatory molecule improves insulin signaling in tissues [31].

The present study demonstrates that circulating levels of IL-12 has a strong relationship with obesity and other parameters of metabolic risk like hypertriglyceridaemia, hyperfibrinogenaemia and sCD40L. Study by Suarez-Alvarez K et al., has also shown that IL-12 has a strong positive relationship with systemic low grade inflammation and obesity related markers [5]. Comparable findings were also reported by Mohamed AA et al., where it was observed that there is increased secretion of proinflammatory cytokines like IL-12 in obese subjects in comparison to normal weight control group [32].

Although, a pathophysiological role cannot be deduced with the study design, the association of circulating cytokines and several anthropometric and metabolic markers linked to insulin resistance points to the participation of these cytokines in the genesis of insulin resistance. The present data suggests that plasma IL-6 and IL-12 values could be used as a metabolic marker in the identification of glucose intolerance and lipid alteration in young obese subjects.

In the present study, the authors observed that fibrinogen was significantly higher in obese individuals, and this marker showed a positive relation with CRP, reflecting the role of underlying subclinical inflammation in obesity. Observation of this study was consistent with study described by Hafez M et al., [33].

It was also observed in the present study that sCD40 L were significantly elevated in obese subjects compared to non obese subjects. Similar to the present study, Unek IT et al., demonstrated that level of CRP and sCD40 L were significantly higher in obese subjects in comparison with normal weight subject [34]. In terms of sCD40 L and cytokines, findings of the present study reveal that, there is a clear positive relationship between these two families. These findings are in tune with a previous study done by El-Shahhat N et al., [35]. As a key player in immunity, CD40 L signaling regulates 'T' cell activation and cytokine production. Besides this, CD40/CD40 L axis is intricately involved in thrombosis and coagulation process [36].

Thus, the present study reveals that a clear positive correlation exists between molecular markers like IL-6, IL-12, sCD40 L with obesity, insulin resistance and prothrombotic factors. This association is a result of close relationship with metabolic pathways and inflammation. Macrophages and adipocytes are directly involved in production of cytokines like IL-6 and IL-12 [37]. Adipocyte hypoxia secondary to adipose tissue expansion leads to development of obesity related inflammation by increasing cytokines production and promotion of expression of proinflammatory genes [38].

As India is presently facing a double threat due to the obesity and diabetes epidemics, urgent measures are necessary to tackle them both. National policies are needed to be formulated to overcome the dual epidemics of diabetes and obesity before they grow out of hand. At present only a few strategies exist to treat obesity. Identifying the emerging role of proinflammatory cytokines in obesity and their relationship with CD40 can throw a light on therapeutic intervention in obesity and mitigate the cardiovascular adverse events.

Evidence suggests that reducing CD40 L by the use of conventional drugs such as statin and antiplatelet agents may lead to improved clinical outcome. Thus, CD40 could be a promising therapeutic target in high risk obese individuals given its close association with atherothrombosis and proinflammatory cytokines [39].

Limitation(s)

The authors acknowledge the following limitations in the present study. First, the sample size was small in the study, hence not empowered to draw a definite conclusion. Second, the authors could not include all the tribes of Tripura, nor could the authors make any subgroup analysis of the different tribes included in the present study. Therefore, results of the present study cannot be extrapolated to other groups. Third, the dietary, occupational and economic parameters of the study population were not categorised in the present study pre specified design. Hence, their social and cultural aspect cannot be commented upon. Finally, as the authors could not follow-up the cases prospectively, incidence of future cardiovascular events remains unknown.

CONCLUSION(S)

In the present study IL-6 and IL-12 had a positive relationship with obesity. Likewise, CD40L is also closely interlinked with the measured cytokines. However, larger clinical studies are required to elucidate all these issues and develop therapeutic strategy of prevention. In absence of a definite therapy to modify the underlying inflammatory and hypercoagulable state, early and more aggressive cardiometabolic risk factor intervention is suggested to mitigate the obesity, one of the greatest public health challenges.

Acknowledgement

At first the authors thank Indian Council of Medical Research, Government of India for giving us permission for this project. The authors are also thankful to the Principal, TMC and DR. BRAM Teaching Hospital for providing all the institutional support. Authors express gratitude to Dr Arjun Das, MD (PSM) and Dr Bitan Sengupta MD (PSM) for helping the statistical analysis.

REFERENCES

[1] Flegal KM, Kit BK, Orpana H, Graubard BI. Association of all cause mortality with overweight and obesity using standard body mass index categories. JAMA. 2013;309(1):71-82.
[2] APCSE. Central obesity and risk of cardiovascular disease in Asia Pacific region. Asia specific Journal of clinical Nutrition. 2006;15(3):287-92.
[3] Piche ME, Lemieux S, Weisnagel SJ, Corneau L, Nadeau A, Bergeron J. Relation of high sensitivity C-reactive protein, interleukin-6, tumour necrosis factor- alpha, and fibrinogen to abdominal adipose tissue, blood pressure and cholesterol and triglyceride levels in healthy postmenopausal women. American Journal of Cardiology. 2005;96 (1):92-97.
[4] Qu D, Liu J, Yu Huang Y. IL-6 in diabetes and cardiovascular complications. British Journal of Pharmacology. 2014;171(15):3595-603.
[5] Suarez-Alvarez K, Solis-Lozano L, Leon-Cabrera S, Gonzalez-Chavez A, Gomez-Hernandez G, Quinones-Alvarez MS, et al. Serum IL-12 is increased in Mexican obese subjects and associated with low-grade inflammation and obesity-related parameters. Mediators of Inflammation. 2013;2013:967067.
[6] De Fronzo RA, Ferrannini E. Insulin resistance: A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia and atherosclerotic cardiovascular disease. Diabetes Care. 1991;14(3):173-94.
[7] Gayoso-Diz P, Otero-González A, Rodriguez-Alvarez MX, Gude F, García F, De Francisco A, et al. Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: Effect of gender and age: EPIRCE cross-sectional study. BMC Endocrine Disorders. 2013;13(1):01-10.

[8] Ernst E. The role of fibrinogen as a cardiovascular risk factor. Atherosclerosis. 1993;100(1):01-12.
[9] Fibrinogen Studies Collaboration, Danesh J, Lewington S, Thompson SG, Lowe GDO, Collins R, et al. Plasma fibrinogen level and risk of major cardiovascular diseases and nonvascular morbidity: An individual participant meta-analysis. JAMA. 2005;294:1799-809.
[10] Santilli F, Basilis, Ferroni P, Davi G. CD-40/CD-40L system & vascular disease. Internal & Emergen of Medicine. 2007;2(4):256-68.
[11] Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Forster R, Müller-Berghaus G, et al. CD-40 Ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature. 1998;391(6667):591-94.
[12] Dechanet J, Grosset C, Taupin JL, Merville P, Banchereau J, Ripoche J, et al. CD-40 Ligand stimulates proinflammatory cytokine production by human endothelial cells. The Journal of Immunology. 1997;159(11):5640-47.
[13] Slupsky JR, Kalbas M, Willuweit A, Henn V, Kroczek RA, Müller-Berghaus G. Activated platelets induce tissue factor expression on human umbilical vein endothelial cells by ligation of CD40. Thrombosis and Haemostasis. 1998;80(12):1008-41.
[14] Zhou L, Stordeur P, De Lavareille A, Thielemans K, Capel P, Goldman M, et al. CD40 engagement on endothelial cells promotes tissue factor-dependent procoagulant activity. Thrombosis and Haemostasis. 1998;79(05):1025-28.
[15] Mishra D, Longkumer I, Saraswathy KN, Devi NK. Obesity and dyslipidemia among Bhil tribal population: A cross-sectional study from India. International Journal of Diabetes in Developing Countries. 2021;23:01-10.
[16] Kandpal V, Sachdeva MP, Saraswathy KN. An assessment study of CVD related risk factors in a tribal population of India. BMC Public Health. 2016;16(1):01-08.
[17] Kshatriya GK, Acharya SK. Triple burden of obesity, undernutrition, and cardiovascular disease risk among Indian tribes. PLoS One. 2016;11(1):e0147934.
[18] Executive summary of the clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. Arch Intern Med. 1998;158(17):1855-67.
[19] Consultation WE. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004;363(9403):157-63.
[20] World Health Organization. Waist circumference and waist-hip ratio: Report of a WHO expert consultation, Geneva, 8-11 December 2008.
[21] Pruden EL. Clinical guide to laboratory tests. Tietz NW, Finley PR, editors. Philadelphia: WB Saunders company; 1995 May 4.
[22] Akbari F, Ghorbani A, Fatehi F. The assessment of proinflammatory cytokines in the patients with the history of cerebral venous sinus thrombosis. Iranian Journal of Neurology. 2016;15(2):75-79.
[23] Haller D, Serrant P, Granato D, Schiffrin EJ, Blum S. Activation of human NK cells by staphylococci and lactobacilli requires cell contact-dependent co-stimulation by autologous monocytes. Clinical and Vaccine Immunology. 2002;9(3):649-57.
[24] Eastham RD. Biochemical Values in Clinical Medicine. 7th Ed. Bristol, England, John Wright & Sons, Ltd; 1985.
[25] Koepke JA, Gilmer Jr PR, Filip DJ, Eckstein JD, Sibley CA. Studies of fibrinogen measurement in the CAP survey program. American Journal of Clinical Pathology. 1975;63(6 Suppl):984-89.
[26] Singh Y, Garg MK, Tandon N, Marwaha RK. A study of insulin resistance by HOMA-IR and its cut-off value to identify metabolic syndrome in urban Indian adolescents. Journal of Clinical Research in Pediatric Endocrinology. 2013;5(4):245-51.
[27] Schoenbeck U, Varro N, Libby P, Buring J, Ridker PM. Soluble CD-40 Ligand and cardiovascular risk in women. Circulation. 2001;104(19): 2266-68.
[28] Roytblat L, Rachinsky M, Fisher A, Greemberg L, Shapira Y, Douvdevani A, et al. Raised interleukin-6 levels in obese patients. Obesity research. 2000;8(9):673-75.
[29] El-Mikkawy DM, EL-Sadek MA, EL-Badawy MA, Samaha D. Circulating level of interleukin-6 in relation to body mass indices and lipid profile in Egyptian adults with overweight and obesity. Egyptian Rheumatology and Rehabilitation. 2020;47(1):01-07.
[30] Baikpour M, Baikpour M, Hosseini M, Sarveazad A. Variations in levels of interleukins and adiponectin in normal and obese adults; a case-control study. Journal of Medical Physiology. 2017;2(2):56-62.
[31] Yeste D, Vendrell J, Tomasini R, Broch M, Gussinye M, Megia A, et al. Interleukin-6 on obese children and adolescences with and without glucose intolerance. Diabetes Care. 2007;30(7):1892-94.
[32] Mohamed AA, Shousha WG, Zaki ME, El-Bassyouni HT, El-Hanafi H, Abdo SM. Inflammatory and endothelial dysfunction indices among Egyptian females with obesity classes I–III. Bioscience Reports. 2020;40(9):BSR20192910.
[33] Hafez M, El-Masry S, Musa N, Fathy M, Hassan M, Hassan N, et al. Relationship between visceral obesity and plasma fibrinogen in obese children. Journal of Pediatric Endocrinology and Metabolism. 2016;29(3):289-96.
[34] Unek IT, Bayraktar F, Solmaz D, Ellidokuz H, Sisman AR, Yuksel F, Yesil S. The levels of soluble CD-40 Ligand and C-reactive protein in normal weight, overweight and obese people. Clinical Medicine & Research. 2010;8(2):89-95.
[35] El-Shahhat N, Ramadan MM, El-Malkey N, Omar AA, Abd El-Aal IA, Eneen A. Soluble CD-40 Ligand, interleukin (IL)-6, and hemostatic parameters in metabolic syndrome patients with and without overt ischemic heart disease. The Egyptian Heart Journal. 2011;63(3-4):131-35.
[36] Daub S, Lutgens E, Münzel T, Daiber A. CD40/CD40L and related signaling pathways in cardiovascular health and disease—the pros and cons for cardio protection. International Journal of Molecular Sciences. 2020;21(22):8533.
[37] Fruhbeck G. The adipose tissue as a source of vasoactive factors. Current Medicinal Chemistry Cardiovascular & Hematological Agents. 2004;2(3):197-208.
[38] Ye J, Gao Z, Yin J, He Q. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. American Journal of Physiology Endocroinology and Metabolism. 2007;293(4):E1118-28.
[39] Semb AG, van Wissen S, Ueland T, Smilde T, Waehre T, Tripp MD, et al. Raised serum levels of soluble CD-40 Ligand in patients with familial hypercholesterolemia: Down regulatory effects of statin therapy. Journal of American College of Cardiology. 2003;41(2):275-79.

PARTICULARS OF CONTRIBUTORS:
1. Professor, Department of Medicine, Tripura Medical College and Dr. BRAM Teaching Hospital, Agartala, Tripura, India.
2. Assistant Professor, Department of Biochemistry, Agartala Government Medical College, Agartala, Tripura, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Partha Sarathi Pal,
Malanchaniwas Qtr, Type-IV/18, Block-8, Near A.G Office, P.O. Kunjavan,
Agartala, Tripura, India.
E-mail: drparthapal65@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Aug 11, 2021
- Manual Googling: Jan 18, 2022
- iThenticate Software: Feb 16, 2022 (25%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Aug 09, 2021**
Date of Peer Review: **Sep 09, 2021**
Date of Acceptance: **Feb 10, 2022**
Date of Publishing: **Mar 01, 2022**