Association of Insulin Resistance with Adipocytokine Levels in Patients with Metabolic Syndrome

Sajimon Thomas · S. Suresh · M. Sudheesh ·
T. Vijayakumar

Received: 23 May 2013 / Accepted: 22 January 2014 / Published online: 5 February 2014
© Association of Clinical Biochemists of India 2014

Abstract Metabolic syndrome (MetS) results from the derangement of adipocyte physiology and carbohydrate metabolism. Obesity and insulin resistance (IR) are integral features of MetS. The adipokine alterations in MetS often correlate with IR and body fat content. High adipose tissue content is associated with a decreased production of adiponectin and excessive production of tumour necrosis factor- α (TNF- α) and interleukin-6 (IL-6), all of which induce IR. The present study evaluated the adipokine alterations in MetS and their association with IR. The findings of the current study indicate that MetS is associated with significant decrease in adiponectin and increase in TNF- α and IL-6. The present study also found that the adipocyte derived inflammatory adipokines, TNF- α and IL-6 correlate with IR while the anti-inflammatory adipokines, adiponectin does not correlate with the degree and severity of IR.

Keywords Metabolic syndrome · Insulin resistance · Adiponectin · Tumor necrosis factor alpha · Interleukin-6

Abbreviations

CVD	Adiponectin, cardio vascular diseases
FBS	Fasting blood glucose
HbA _{1c}	Glycated hemoglobin
IR	Insulin resistance
IL-6	Interleukin-6
MetS	Metabolic syndrome
TNF- α	Tumor necrosis factor alpha
T2DM	Type 2 diabetes mellitus

Introduction

Metabolic syndrome (MetS) is a widely prevalent and multi-factorial disorder that presents in a distinct, albeit heterogeneous phenotype. It is a cluster of disorders that occur together, increasing the risk of heart disease, stroke and type 2 diabetes mellitus (T2DM). The disorders include elevated blood pressure, insulin resistance (IR), and excess body fat around the waist and/or dyslipidemia. The prevalence of MetS depends on age, ethnic background, and gender. It rises linearly from 20 to 50 years and plateaus thereafter [1]. It is estimated that a large majority (~75 %) of patients with T2DM or impaired glucose tolerance (IGT) have MetS. The presence of MetS in these populations relates to higher prevalence of cardio vascular disease (CVD) compared to patients with T2DM or IGT without the syndrome [2]. T2DM is the most common form and is characterized by disorders of insulin action and secretion, IR with relative insulin deficiency or predominantly as insulin secretory defect with or without IR [3]. Incidence of T2DM increases steadily with age from about 6 % of the total population to about 11 % by

S. Thomas · M. Sudheesh
Dianova Laboratories, Gandhinagar, Kottayam, Kerala 686 008,
India

S. Suresh (✉)
Department of Biochemistry, School of Medical Education,
Mahatma Gandhi University, Gandhinagar, Kottayam,
Kerala 686 008, India
e-mail: sureshkrishnakadambari@gmail.com

T. Vijayakumar
Department of Basic Medical Sciences, Educare Institute of
Dental Sciences, Malappuram, Kerala, India
e-mail: tvkumarvarkala@gmail.com

the age of 65. People with T2DM are frequently resistant to the action of insulin [4]. In patients with MetS the incidence of coronary disease and carotid atherosclerosis is higher along with higher mortality from all such causes [5]. Some studies had shown that, regardless of the criteria used obesity was the most common characteristic of MetS and it was also suggested that obesity should be considered as an essential component of the set of cardiovascular risk factors in order to identify subjects with a higher risk of co morbidities and early CVD [6]. The degree of obesity was reported to influence the onset of alterations that comprise MetS increasing the risk of cardio-MetS [7].

Insulin resistance was reported to be the most important single risk factor predisposing an individual to MetS and all the known associated complications of the syndrome. IR can be defined as an insufficient response of target organs such as liver, skeletal muscle and adipose tissue, to the physiological plasma insulin levels and a disruption in the insulin signalling transduction pathway downstream the insulin receptor, and is considered to be the most important underlying mechanism of MetS [8]. IR, or its accomplice, hyperinsulinemia, is the main determinant of metabolic risk, independent of age [9]. Obesity had been shown to be the most important and independent etiological factor as the commonest symptom of MetS [10]. Several studies had revealed that the plasma concentration of inflammatory markers or mediators, such as TNF- α , IL-6, hs-CRP, fibrinogen, and plasminogen activator inhibitor-1 (PAI-1) levels were increased in the insulin-resistant states of obesity and T2DM [11, 12]. In obesity, the expression, synthesis and release of the pro inflammatory adipokines such as TNF- α , IL-6, leptin and resistin are enhanced; but that of anti inflammatory molecules such as adiponectin is decreased. Many of these adipokines such as adiponectin,

Materials and Methods

A cross-sectional, case-control study was conducted at the Department of Physiology and Biochemistry, Educare Institute, Malappuram, in association with the Department of Medicine, Medical College Hospital, Kottayam, Kerala. Both the MetS and control subjects (men and women) were included after getting the informed consent as per the criteria laid down by the Institutional Ethics Committee. Two hundred and fifty subjects in the age group of 31–50 years with MetS as per the revised criteria of the International Diabetes Federation (IDF) formed the MetS group [19]. One hundred and fifty healthy age and sex matched control subjects were selected from the siblings and staff of the hospital. None of the subjects were suffering from any acute or chronic illness and were not on any treatment.

Blood samples of all subjects were collected after 12 h fasting. Plasma and serum were used for the following investigations and the data so obtained were documented. Glucose was estimated using Randox- Daytona Fully Automated Biochemistry Analyzer. Insulin and C-peptide were assayed by Chemi Luminescent Immuno Assay (CLIA) in Advia Centaur-fully automated CLIA analyzer of M/s. Siemens Health Care Diagnostics India Ltd. Glycated hemoglobin (HbA_{1c}) was estimated by HPLC using Bio-Rad D 10 Analyzer. TNF- α and IL-6 were analyzed by CLIA using Immulite 1000 (M/s. Siemens Health Care, Germany). Adiponectin was assayed by using ELISA kit manufactured by Otsuka Pharmaceuticals, Japan in Biorad 680 micro plate reader [20–23]. Quality control (QC) was performed by participating in the BIO-RAD EQUAS international QC programmes. IR determination was by the homeostasis model assessment for IR (HOMA-IR). The calculation of IR was by employing the following formula [24].

$$\text{IR} = \frac{\text{Fasting blood glucose (mg/dl)} \times \text{Fasting plasma insulin (}\mu\text{U/ml)}}{405}$$

TNF- α , IL-6, fetuin-A, resistin, visfatin, leptin etc. are well characterized and their association with IR, obesity and MetS were reported [13, 14]. Eventhough several adipokines were implicated to play a role in the pathogenesis of MetS, adiponectin, TNF- α and IL-6 are the most important in the pathogenesis of the syndrome [15, 16]. Adiponectin enhances insulin sensitivity in liver and stimulates glucose uptake and fatty acid oxidation in muscles. This will also explain IR observed in patients with MetS [17, 18].

Data from the above investigations were statistically analyzed employing the Holm-Sidak method of multiple pairwise comparison procedures of One Way Anova test. Pearson product moment correlation test was used for correlation analysis between a dependent variable and an independent variable. The overall critical confidence level of the present study was 95 % (α -0.05 and β -0.95) and hence, results with p values < 0.05 were interpreted as statistically significant [25]. All statistical procedures and tests were conducted

Table 1 Glycemic indices of MetS patients and healthy control subjects

Subjects	Age groups	Gender	Group	Glycemic indices	
				FBS (mg/dl)	HbA1c (%)
Control	31–40	Men	I (a)	88.65 ± 8.787	5.03 ± 0.281
		Women	I (b)	86.32 ± 7.541	4.94 ± 0.362
	41–50	Men	II (a)	92.43 ± 9.563	5.67 ± 0.413
		Women	II (b)	90.75 ± 6.814	5.13 ± 0.402
Test	31–40	Men	III (a)	143.10 ± 17.035	6.08 ± 0.383
		Women	III (b)	141.98 ± 15.674	5.75 ± 0.387
	41–50	Men	IV (a)	146.53 ± 18.761	6.44 ± 0.474
		Women	IV (b)	144.29 ± 16.921	5.97 ± 0.496

All values are expressed as Mean ± SD

Table 2 One Way Anova test statistics of glycemic indices

Comparisons	DF	FBS		HbA1c	
		$F_{\alpha 0.05}$	p	$F_{\alpha 0.05}$	p
I (a) versus III (a)	67	241.371	<0.001*	153.632	<0.001*
I (b) versus III (b)	136	295.041	<0.001*	138.451	<0.001*
III (a) versus III (b)	132	0.138	0.711	20.820	<0.001*
II (a) versus IV (a)	73	212.020	<0.001*	52.525	<0.001*
II (b) versus IV (b)	116	423.996	<0.001*	93.883	<0.001*
IV (a) versus IV (b)	114	0.443	0.507	25.588	<0.001*
III (a) versus IV (a)	84	0.782	0.3791	4.826	<0.001*
III (b) versus IV (b)	162	0.811	0.369	10.184	<0.01*

DF Degrees of freedom, $F_{\alpha 0.05}$ F value at 95 % confidence level

* Statistically significant 'p' values are indicated by an asterisks mark

using Sigma Stat 6.5 Version Software (M/s. Sigma-Aldrich Co., St. Louis, USA).

Results

Tables 1 and 2 show that FBS and HbA_{1c} levels of men and women in both the age groups were significantly elevated in the MetS group, as compared to their age-matched control groups ($p < 0.001$). A significant rise in FBS of men was observed in the 31–40 age MetS group, as compared to women of the same age group ($p < 0.001$). But such an alteration in FBS of men of the 41–50 age MetS group was not observed compared to the age-matched MetS group women. The FBS of the upper age group men and women showed no change compared to the corresponding lower age group. HbA_{1c} levels of men in both the 31–40 and 41–50 age groups were increased significantly ($p < 0.001$) compared to the age-matched women subjects ($p < 0.001$). The HbA_{1c} levels of men and women in the 41–50 age group were significantly increased than that of their respective 31–40 age group ($p < 0.001$ and <0.01).

Tables 3 and 4 show that plasma insulin and C-peptide levels of the MetS group men and women in both the age-

Table 3 Levels of insulin and C-peptide in plasma and IR of MetS patients and healthy control subjects

Group	Insulin (uIU/ml)	C-peptide (ng/ml)	IR
I (a)	3.15 ± 1.099	0.525 ± 0.0450	0.7 ± 0.024
I (b)	2.97 ± 1.124	0.501 ± 0.0362	0.6 ± 0.021
II (a)	3.62 ± 1.331	0.647 ± 0.0513	0.83 ± 0.031
II (b)	3.47 ± 1.452	0.632 ± 0.0421	0.78 ± 0.024
III (a)	9.42 ± 1.117	0.963 ± 0.0582	3.33 ± 0.047
III (b)	8.97 ± 1.884	0.911 ± 0.0640	3.14 ± 0.072
IV (a)	9.16 ± 1.681	0.945 ± 0.0537	3.31 ± 0.078
IV (b)	9.02 ± 1.524	0.933 ± 0.0528	3.21 ± 0.064

All values are expressed as Mean ± SD

Table 4 One Way Anova test statistics of of Insulin, C-peptide and IR. Degrees of freedom as in Table 2

Comparisons	Insulin		C-peptide	
	$F_{\alpha 0.05}$	p	$F_{\alpha 0.05}$	p
I (a) versus III (a)	531.072	<0.001	1123.743	<0.001
I (b) versus III (b)	388.541	<0.001	1595.665	<0.001
III (a) versus III (b)	2.020	0.158	19.819	<0.001
II (a) versus IV (a)	229.521	<0.001	574.250	<0.001
II (b) versus IV (b)	389.293	<0.001	1074.207	<0.001
IV (a) versus IV (b)	0.214	0.644	1.404	0.239
III (a) versus IV (a)	0.699	0.405	2.224	0.140
III (b) versus IV (b)	0.0333	0.855	5.519	<0.05

groups were significantly elevated as compared to their age-matched controls ($p < 0.001$). Both plasma insulin and C-peptide levels of men in the 31–40 age group were significantly higher than that of the age-matched women irrespective of MetS or control group ($p < 0.001$), but such a change was not observed in men and women of the 41–50 years age group. The plasma insulin levels of the upper age group men and women were not increased significantly as compared to the corresponding lower age group while, the plasma C-peptide levels of the upper age

Table 5 Adipokine levels of MetS patients and healthy control subjects

Group	Adiponectin (μg/ml)	TNF-α (pg/ml)	IL-6 (pg/ml)
I (a)	6.83 ± 0.99	5.86 ± 1.33	11.93 ± 2.14
I (b)	8.11 ± 1.34	5.17 ± 1.04	10.56 ± 1.89
II (a)	6.45 ± 0.93	6.24 ± 1.45	13.47 ± 2.82
II (b)	7.84 ± 1.22	5.93 ± 1.12	12.18 ± 2.34
III (a)	5.92 ± 0.92	6.75 ± 1.41	16.27 ± 2.88
III (b)	7.38 ± 0.98	6.03 ± 1.19	14.95 ± 2.23
IV (a)	5.19 ± 0.86	7.35 ± 1.62	18.91 ± 3.24
IV (b)	6.93 ± 0.95	6.87 ± 1.31	16.78 ± 2.66

All values are expressed as Mean ± SD

group women was increased when compared to the women of the lower age MetS group ($p < 0.05$), while such a change was not observed between the plasma C-peptide levels of men in the 31–40 and 41–50 age groups.

Adiponectin values are given in Tables 5 and 6. Adiponectin values of the MetS patients were statistically lower than that of the control subjects of the corresponding age groups. The values were found to be decreasing with increasing age both in control and MetS groups. Adiponectin levels of the women in the MetS and control groups were significantly higher than the

corresponding men. The statistical analysis of the TNF-α value revealed significant elevation with increase in age of both in men and women of the control and MetS group groups. Comparison of the TNF-α value between men and women of the two different age groups was significant in all cases except men and women of the test group between 41 and 50 years. The values were significantly higher in both the men and women of the MetS group compared to the corresponding control subjects. The IL-6 values of the MetS group were statistically higher than that of the corresponding control group. The values were found to be increasing with increase in age both in men and women of the MetS and control groups. These differences were found to be statistically significant. Comparison between men and women also revealed significant difference. Table 7 shows that adiponectin is not correlated with either the glycemic indices (except HbA_{1c}), or IR, whereas TNF-α and IL-6 show significant correlations with the above parameters.

Discussion

The presence of MetS is found to be associated with increased risk of T2DM or IDF defined MetS. T2DM is characterized by disorders of insulin action and secretion,

Table 6 One Way Anova test statistics of adipokines. DF as in Table 2

Comparisons	Adiponectin		TNF-α		IL-6	
	F _α 0.05	<i>p</i>	F _α 0.05	<i>p</i>	F _α 0.05	<i>p</i>
I (a) versus III (a)	15.304	<0.001	6.937	<0.05	46.103	<0.001
I (b) versus III (b)	13.132	<0.001	12.787	<0.001	101.491	<0.001
III (a) versus III (b)	65.513	<0.001	6.244	<0.05	5.703	<0.05
II (a) versus IV (a)	36.202	<0.001	9.175	<0.01	67.890	<0.001
II (b) versus IV (b)	20.636	<0.001	16.300	<0.001	92.903	<0.001
IV (a) versus IV (b)	99.317	<0.001	3.071	0.082	20.254	<0.001
III (a) versus IV (a)	14.463	<0.001	4.168	<0.05	20.175	<0.001
III (b) versus IV (b)	8.716	<0.01	11.412	<0.01	14.725	<0.001

Table 7 Pearson product moment correlation statistics of adipokines with the glycemic indices and IR in MetS patients

Independent variables	Insulin	C-peptide	Glucose	HbA _{1c}	IR
Adiponectin					
<i>r</i>	−0.557	−0.592	−0.564	−0.820	−0.564
<i>p</i>	0.151	0.122	0.145	<0.05*	0.145
TNF-α					
<i>r</i>	0.768	0.825	0.782	0.931	0.774
<i>p</i>	<0.05*	<0.05*	<0.05*	<0.001*	<0.05*
IL-6					
<i>r</i>	0.900	0.925	0.913	0.977	0.905
<i>p</i>	<0.01*	<0.01*	<0.01*	<0.001*	<0.01*

r correlation coefficient

* Statistically significant
p values are indicated by an asterisk mark

IR with relative insulin deficiency or predominantly as insulin secretory defect with or without IR. Individuals with T2DM have relative insulin deficiency. Even in the absence of IR obesity was found to be associated with increased risk of T2DM. However, the presence of MetS was found to be associated with an increased risk for T2DM regardless of the anthropometric indices. The observed alterations in glycemic indices this study in subjects with MetS suggests that these subjects have peripheral IR. Further, this finding substantiates the association between MetS and the development of T2DM. IR leads to a compensatory increased production of insulin by the pancreatic beta-cells resulting in hyperinsulinemia. IR is the main determinant of the metabolic risk, independent of age. The finding of the present study is consistent with other studies highlighting the importance of IR in MetS [5].

Several factors appear to be involved in the development of IR. Obesity is one of the most common finding in MetS, and the excess adipose tissue, particularly visceral adipose tissue is a source of several molecules such as adipocytokines (adipokines) that induce IR. The adipokines produced by adipocytes are involved in energy metabolism [8]. In obesity, there is enhanced adipocyte production of several inflammatory cytokines such as CRP, TNF- α , leptin and IL-6. TNF- α and IL-6 contribute to decreased insulin-sensitivity in adipocytes, liver and skeletal muscles, by impairing insulin signal transduction. Excessive adipose tissue is also associated with a decreased production of adiponectin, a molecule that is found to have anti-diabetic, anti atherosclerotic and anti-inflammatory functions [17, 18]. The imbalanced production of pro- and anti-inflammatory cytokines by adipocytes not only creates the inflammatory *milieu* associated with obesity but also decreases insulin-sensitivity in various target organs by impairing insulin signal transduction [6–9]. Several authors had shown that adipose tissue dysfunction is related to its components or MetS itself by a cause-effect relationship [10–14]. In the present study, significant decrease in the level of adiponectin and increase in the levels of TNF- α and IL-6 were observed in subjects with MetS compared to their age and sex matched counter parts.

The correlation of TNF- α with obesity and IR, were reported earlier [7, 8]. Similarly, IL-6 is also reported to be elevated in patients with MetS [9–14]. The elevation in TNF- α and IL-6 reported to be associated with IR, pro inflammatory and pro thrombotic changes. This may result in increased prevalence of chronic degenerative diseases such as T2DM, hypertension and CVD [15, 16]. Along with this, the decreased level of adiponectin, a potent anti inflammatory cytokine will further augment the risk of chronic degenerative diseases. TNF- α and IL-6 showed a positive correlation with IR whereas adiponectin and IR were negatively correlated, which is statistically not

significant. This observation of the present study underlines the fact that the levels of the inflammatory adipokines, TNF- α and IL-6 are dependent on IR whereas that of adiponectin is independent of IR in MetS patients. The seemingly paradoxical finding that there exists no correlation between IR and adiponectin levels might be attributed to the fact that in the context of this study, adiponectin levels *per se* correlate with the number and size of adipocytes (or the degree of adiposity) rather than with IR. This is documented by similar studies elsewhere, which also reported non-significant or casual associations of IR with adiponectin levels in MetS patients [26, 27].

Conclusions

MetS, a life style disease is associated with alterations in glycemic indices and adipokines levels, which predispose an individual to T2DM and CVD. In the present study an attempt was being made to correlate the adipokines levels with the IR of individuals having MetS. The glycemic indices (FBS, HbA1c, insulin and C-peptide) of the subjects in the MetS group was significantly higher than that of their normal counter parts, both in men and women. The inflammatory adipokines (TNF- α and IL-6) were increased whereas the anti-inflammatory adipokine, adiponectin was decreased in subjects with MetS. The inflammatory adipokines showed positive correlations with IR while the anti-inflammatory adipokine, adiponectin did not show a significant negative correlation with IR in patients with MetS. So it could be concluded from the present study that there were elevations in FBS, HbA1c, insulin, C-peptide, IR, TNF- α and IL-6 which predispose people to the risk of chronic degenerative conditions like CVD, CHD or T2DM as compared to their age-matched controls. On the contrary, adiponectin, a parameter which is associated with a decreased risk for these diseases tend to fall significantly in MetS patients than their normal counter parts. Moreover, serum levels of the inflammatory adipokines correlate with IR in both men and women across different age groups with MetS.

References

1. Cameron AJ, Shaw JE, Zimmet PZ. The metabolic syndrome: prevalence in worldwide populations. *Endocrinol Metab Clin North Am.* 2004;33:351–75.
2. Prabhakaran D, Anand SS. Metabolic syndrome: an emerging risk state for cardiovascular disease. *Vasc Med.* 2004;9:55–68.
3. Lorenzo C, Williams K, Hunt KJ, Haffner SM. Trend in the prevalence of metabolic syndrome and its impact on cardiovascular disease incidence: the San Antonio Heart Study. *Diabetes Care.* 2006;29:625–30.

4. Roger VL, Go AS, Jones LDM, Adams RJ, Berry JD, Brown TM, et al. On behalf of the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2011 Update: a report from the American Heart Association. *Circulation*. 2011;123:18–209.
5. Han TS, Williams K, Sattar N, Hunt KJ, Lean MEJ, Haffner SM, et al. Analysis of obesity and hyperinsulinemia in the development of metabolic syndrome: San Antonio Heart Study. *Obes Res*. 2002;10:923–31.
6. Kotani K, Sakane N. Leptin: Adiponectin ratio and metabolic syndrome in the general Japanese population. *Korean J Lab Med*. 2011;31:162–6.
7. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome and risk of incident cardiovascular events: an eight year follow up of 14,719 initially healthy America women. *Circulation*. 2003;107:391–7.
8. Koerner A, Kratzsch J, Kiess W. Adipocytokines: leptin-the classical, resistin-the controversial, adiponectin-the promising, and more to come. *Best Pract Res Clin Endocrinol Metab*. 2005;19:525–46.
9. Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Pader DJ, et al. Resistin is an inflammatory marker of atherosclerosis in humans. *Circulation*. 2005;111:932–9.
10. Matsushita K, Yatsuya H, Tamakoshi K, Wada K, Otsuka R, Takefuji S, et al. Comparison of circulating adiponectin and proinflammatory markers regarding their association with metabolic syndrome in Japanese men. *Arterioscler Thromb Vasc Biol*. 2006;26:871–6.
11. Whitehead JP, Richards AA, Hickman IJ, Macdonald GA, Prins JB. Adiponectin- a key adipokine in the metabolic syndrome. *Diabetes Obes Metab*. 2006;8:264–80.
12. Stevuljevic KJ, Memon L, Stefanovic A, Spasic S, Kalimanovska VS, Stanojevic NB, et al. Correlation of oxidative stress parameters and inflammatory markers in coronary artery disease patients. *Clin Biochem*. 2007;40:181–7.
13. Renaldi O, Pramono B, Sinorita H, Punomo LB, Asdie RH, Asdie AH, et al. Hypoadiponectinemia: a risk factor for metabolic syndrome. *Acta Med Indones*. 2009;41:20–34.
14. Wood IS, Wang B, Trayhurn P. IL-33, a recently identified interleukin-1 gene family member, is expressed in human adipocytes. *Biochem Biophys Res Commun*. 2009;384:105–9.
15. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Oh-sugi M, et al. CD8+effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat Med*. 2009;15:914–20.
16. Troseld M, Seljeflot I, Arnesen H. The role of interleukin-18 in the metabolic syndrome. *Cardiovasc Diabetol*. 2010;9:467–82.
17. Terra X, Quintero Y, Auguet T, Porras JA, Hernandez M, Sabench F, et al. FABP 4 is associated with inflammatory markers and metabolic syndrome in morbidly obese women. *Eur J Endocrinol*. 2011;164:539–47.
18. Sharma P. Inflammation and the metabolic syndrome. *Ind J Clin Biochem*. 2011;26:316–7.
19. IDF (International Diabetes Federation), *Diabetes Atlas 4th ed*. 2009, Brussels.
20. Everse SJ, Spraggon G, Veerapandian L, Reley M, Doolittle RF. Crystal structure of fragment double-D from human fibrin with two different bound ligands. *Biochemistry*. 1988;27:8637–42.
21. Kaplan LA, Pesce AJ. *Clinical chemistry: theory, analysis, and correlations*. 3rd ed. St. Louis: CV Mosby; 1996. p. 619–81.
22. Clark PM. Assays for insulin, proinsulin(s) and C-peptide. *Ann Clin Biochem*. 1999;36:541–64.
23. Burtis CA, Ashwood ER, Bruns DE. *Carbohydrates: methods for determination of glycated hemoglobins*; in Tietz text book of clinical chemistry and molecular diagnostics. 4th ed. Philadelphia: WB Saunders; 2004. p. 396–405.
24. Emoto M, Nishizawa Y, Maekawa K, Hiura Y, Kanda H, Kawagishi Y, et al. Homeostasis model assessment as a clinical index of insulin resistance in type 2 diabetic patients treated with sulfonylureas. *Diabetes Care*. 1999;22:818–22.
25. Mahajan BK. *Methods in biostatistics for medical students and research workers*. 7th ed. New Delhi: Jaypee Brothers; 2010. p. 80–194.
26. Gao H, Fall T, van Dam RM, Flyvbjerg A, Zethelius B, Ingelsson E, Hagg S. Evidence of a causal relationship between adiponectin levels and insulin sensitivity: a Mendelian randomization study. *Diabetes*. 2013;62:1338–44.
27. Abdelgadir M, Karlsson AF, Berglund L, Berne C. Low serum adiponectin concentrations are associated with insulin sensitivity independent of obesity in Sudanese subjects with type 2 diabetes mellitus. *Diabetol Metabol Syndr*. 2013;5:1–5.