

# Chronic sub-clinical inflammation in the abdominal adipose tissue – Evaluation of inflammatory cytokines and their link with insulin resistance in metabolically obese South Indians: A cross-sectional observational study

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### ABSTRACT

**Objective:** To measure the levels of proinflammatory cytokines tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), and high-sensitive C-reactive protein (hs-CRP) and the anti-inflammatory cytokine adiponectin (AN) in obese South Indian subjects and to ascertain whether or not a causal role could be ascribed to these cytokines in the development of insulin resistance (IR).

**Materials and Methods:** Forty obese and forty nonobese volunteers of both genders were recruited. Parameters such as body mass index (BMI), waist circumference (WC), and blood pressure were evaluated. Fasting blood sugar (FBS), fasting insulin level, hemoglobin A1c (HbA1C), lipid profile, TNF- $\alpha$ , IL-6, hs-CRP, and AN levels were measured. IR was evaluated by homeostatic model assessment-IR method. Abdominal adiposity was measured by ultrasonography. The results were statistically evaluated by appropriate tests. **Results:** BMI, WC, and visceral fat were high in the obese group. Females had higher subcutaneous fat in both groups. HbA1C was marginally high in the obese group ( $P = 0.014$ ). IR was high in all the groups, obese males showing higher values (not significant [NS]). Total cholesterol and low-density lipoprotein were high in the obese group ( $P = 0.028$ ,  $P = 0.003$ ). TNF- $\alpha$  was high in obese males (NS), IL-6 was high in both groups, higher in nonobese females (NS), hs-CRP was high in both groups, higher in females of both groups (NS). AN was high in females of both groups ( $P = 0.002$ ). **Conclusions:** In this study on South Indian subjects, proinflammatory cytokines such as IL-6 and hs-CRP, despite being high, did not show any causal correlation either with abdominal obesity or with IR. TNF- $\alpha$  being normal showed some correlation which was inconsistent. Even the anti-inflammatory adipokine, AN did not show any correlation with IR. Cytokines had an inconsistent correlation with the components of metabolic syndrome hence were not useful.

**Key words:** Abdominal adiposity, adiponectin, inflammatory cytokines

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## INTRODUCTION

Adipose tissue has attracted a great deal of attention as a pathogenic site for obesity-induced insulin resistance (IR). All adipose tissues are not equal, with visceral fat (VF) being more pathogenic.<sup>[1]</sup> High rates of lipolysis in the visceral adipose tissue causing an increase in free fatty acids and their ectopic deposition in the skeletal muscle and liver was proposed as the reason for skeletal muscle and hepatic IR.<sup>[2-4]</sup> Increased visceral adipose tissue was thought to be the conduit by which obesity lead to IR.<sup>[5]</sup>

The mild chronic inflammation in the adipose tissue especially in the VF, with increased secretion of proinflammatory adipocytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), high-sensitive C-reactive protein (hs-CRP), may be the important link connecting visceral adiposity with IR.<sup>[5-9]</sup> Although it is debated as to how TNF- $\alpha$  and IL-6 expression induce IR, both have been demonstrated to interfere with insulin signaling in the adipose tissue and the liver.<sup>[9-12]</sup> CRP is an acute phase reactant and is produced in the liver, in response to IL-6 and is considered as a marker for IR.<sup>[13-15]</sup> and abdominal obesity is a critical correlate of CRP.<sup>[16]</sup> Adiponectin (AN) is an adipokine produced by the VF and is reduced in those who are obese and having IR, and is predictive of future diabetes.<sup>[17,18]</sup>

In a nutshell, proinflammatory and anti-inflammatory adipokines and adipocytokines are produced by visceral adipose tissue and their dysregulation lead to chronic low-grade inflammatory state and contribute to the development of IR.<sup>[12,19]</sup>

In our previous study, the Mysore Visceral Adiposity in Diabetes study (MYVAD)<sup>[20]</sup> we attempted to correlate visceral and subcutaneous fat (SCF) with IR in obese and nonobese, diabetic and nondiabetic individuals and to our surprise, found no correlation at all. We expressed that there had to be other causes for IR in these individuals and in our quest to find out for other causes, we have estimated, TNF- $\alpha$ , IL-6, hs-CRP, and AN along with fasting blood sugar (FBS), fasting insulin levels (FILs), HbA1C, lipid profile, in the obese nondiabetic and nonobese nondiabetic individuals, the latter acting as controls to find out answers to the objectives of this study.

### Study goals and objectives

#### Primary

To find out the levels of proinflammatory cytokines such as TNF- $\alpha$ , IL-6, and hs-CRP in those having visceral adiposity and IR and to know whether they correlate with VF and SCF to establish a causal link for IR.

#### Secondary

(a) To know whether these cytokines vary in those who have IR compared to those without IR, irrespective of whether they are metabolically obese or not. (b) To ascertain the significance of AN in the causation of IR. (c) Whether cytokines correlate with the components of metabolic syndrome.

## MATERIALS AND METHODS

This study is a prospective, cross-sectional, comparative, and observational study done on subjects recruited from the clinics of the four investigators. The study period was from October 2013 to March 2014. We recruited 80 subjects, 40 being obese nondiabetics, treated as a test sample, and 40 being nonobese nondiabetics, treated as controls. Ethical clearance was obtained from the Institutional Ethical Committee of JSS Medical College, Mysore. Informed consent was obtained from each individual participant subject.

#### Inclusion criteria

Healthy adults aged between 18 and 65 years who fulfilled the criteria of normal and obese according to the criteria laid down for Asian Indians.<sup>[21]</sup>

#### Exclusion criteria

(1) Type 1 diabetes, (2) Type 2 diabetics, (3) presence of any acute illness, (4) pregnancy, (5) subjects on anti-obesity medication, (6) comorbid conditions such as chronic obstructive pulmonary disease, HIV, and tuberculosis, and (7) subjects coming under the category of overweight (body mass index [BMI] of 23–24.9 kg/m<sup>2</sup>).

Height, in centimeters to the nearest cm, and weight in kilograms, to the nearest 100 g were recorded. Waist circumference (WC) was measured (cm) midway between the lower border of the ribs and the iliac crest with the subject in standing position. Blood pressure (BP) was recorded in the sitting position, in the right arm, with a mercury sphygmomanometer (ISI certified instrument) after 5 min rest. Average of three readings was taken. FBS (GOD-PAP method), hemoglobin A1c (HbA1C) (high-performance liquid chromatography method), serum cholesterol (CHOD-PAP method), serum triglycerides (enzymatic method), high-density lipoprotein (HDL) (3<sup>rd</sup> generation direct assay) low-density lipoprotein (LDL) (3<sup>rd</sup> generation direct assay), and serum insulin fasting assay (chemiluminescence immunoassay method), were done at the National Accreditation Board for Testing and Calibration Laboratories accredited standard laboratory. Blood collected there was stored at –20°C. Once the blood collection from all the 80 subjects, was over, it

was sent under appropriate cold storage conditions, to the Biochemistry Laboratory of JSS Medical College, Mysore, for estimating TNF- $\alpha$ , IL-6, hs-CRP, and AN. IR was calculated by the homeostatic model assessment (HOMA)-IR assessment formula of FBS (m moles) multiplied by fasting insulin (mIU) divided by 22.5.<sup>[22]</sup>

### Biochemical investigations

Serum levels of IL-6 (Diclone, France), TNF- $\alpha$  (Diclone, France), and AN (Assaypro cat no. EA25001) were determined by double antibody method enzyme-linked immunosorbent assay (ELISA) kits. As per the kit manufacturer's instructions, using automated ELISA reader and washer (Bio-Rad, USA) and samples for hs-CRP was analyzed using IMOLA biochemistry analyzer by immunoturbidimetry method (Randox, USA). Test for hs-CRP had a sensitivity of 0.477 mg/L and an intra-assay precision of 4.91–1.21% and interassay precision of 4.98–1.22%. TNF- $\alpha$  and IL-6 had a sensitivity of 8.0 pg/ml and 2.0 pg/ml, respectively and an intra-assay and interassay precision of 3.3% and 9.0% for TNF- $\alpha$  and 4.9% and 7.7% for IL-6. AN had a sensitivity to detect the concentration of <0.7 ng/ml and the test had an intra-assay precision of 4.3% and interassay precision of 7.2%.

### Sonographic measurements

The measurement of SCF, preperitoneal fat (PPF) and VF was done by the same ultrasonologist for all subjects using a GE P5 logic system with multiple frequencies (2–5 MHz) convex probe for measuring VF and linear probe (8–12 MHz) for measuring abdominal wall fat. Criteria, as defined by Stolk *et al.*<sup>[23]</sup> was used for the measurement, the details are as given below.

VF thickness (defined as the distance between the anterior border of lumbar vertebra and posterior surface of rectus abdominus muscle) was measured midway between xiphisternum and umbilicus, approximately 5 cm from umbilicus at three positions along the horizontal line [Figure 1]. All measurements were done at the end of quiet expiration, applying minimal pressure, not displacing or deforming the abdominal contents.<sup>[23]</sup>

Longitudinal scans were obtained using a linear probe along the midline (linea alba) and fat skin barrier. The thickness of the SCF was defined as the distance between the anterior surface of the linea alba and the fat skin barrier. PPF was measured as extending from the anterior surface of the left lobe of the liver to the posterior surface of the linea alba [Figure 2]. Fat deposits in the liver were also graded.

### Statistical analysis

Statistical methods such as descriptive statistics, two-way ANOVA, and product moment correlations were applied

for the present study. Two-way ANOVA was applied to find out the influence of obese versus no obese with gender for the parameters selected. Correlation coefficients were applied to find out the mutual relationship between selected parameters. The significance levels fixed for 0.05 levels for all the statistical tests applied. All the statistical operations were done through SPSS for windows (version 16.0).

## RESULTS

Of the 80 subjects recruited, there was one drop out, out of whom 36 (45.5%) were in the obese group, and 43 (54.5%) were in the nonobese group. The mean age of the participants in the two groups were well matched ( $36.45 \pm 11.3$  years, and  $36.43 \pm 10.79$  years). There were 20 males and 16 females in the obese group and 31 males and 12 females in the nonobese group. The mean age of males in both the groups was almost the same whereas nonobese females ( $31.45 \pm 10.74$  years) were much younger than obese females ( $40.09 \pm 7.51$  years) [Tables 1 and 2].

The physical characteristics, the values of abdominal fat measured by ultrasonography, values of components of metabolic syndrome, and of the cytokines are shown in Table 3a and b. Males had lower BMI but higher WC, whereas females had higher BMI but lower WC. Group comparisons were significant ( $P = 0.000$ ). Males had higher VF and females had higher SCF in both groups, and all the values were statistically significant ( $P = 0.000$ ). Systolic BP (SBP) was marginally higher in the obese group ( $P = 0.023$ ), and diastolic BP (DBP) was similar in both groups and genders. The mean HbA1C was marginally higher in the obese group ( $P = 0.014$ ). There was no statistically significant difference, in FBS, FIL, and IR in both groups and genders. Total cholesterol (TC) was marginally high in the obese group ( $P = 0.028$ ). TG was significantly lower in females of both groups ( $P = 0.01$ ).

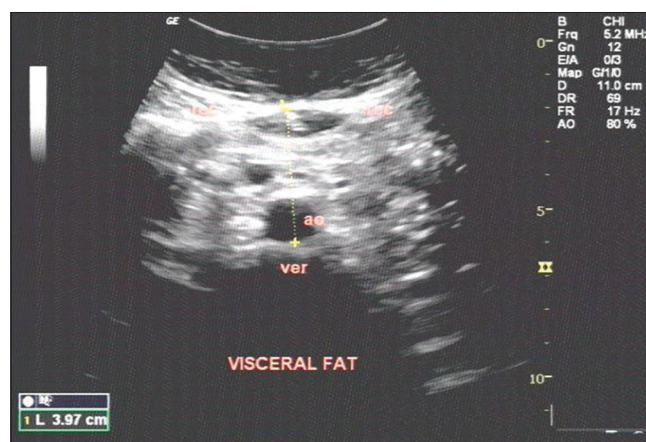


Figure 1: Measurement of visceral fat by ultrasound



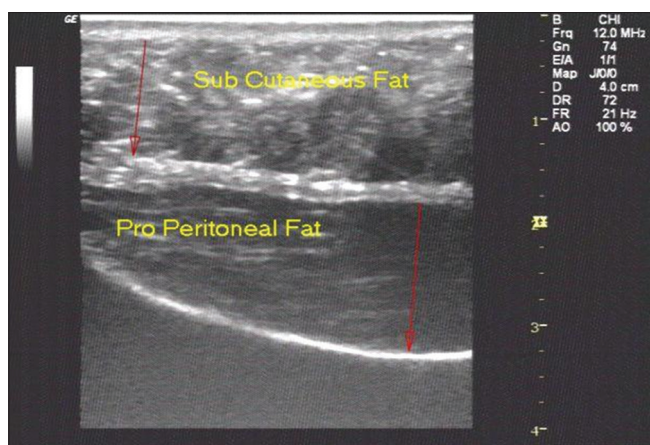


Figure 2: Measurement of subcutaneous fat by ultrasound

Table 1: Age group of the participants

Crosstab	Group		Total
	Obese	Nonobese	
Ages			
<20			
Count	0	3	3
Percentage of group	0.0	7.0	3.8
21-30			
Count	9	15	24
Percentage of group	25.0	34.9	30.4
31-40			
Count	13	12	25
Percentage of group	36.1	27.9	31.6
41-50			
Count	10	11	21
Percentage of group	27.8	25.6	26.6
50+			
Count	4	2	6
Percentage of group	11.1	4.7	7.6
Total			
Count	36	43	79
Percentage of group	100.0	100.0	100.0

Table 2: Gender analysis of the participants

Crosstab	Group		Total	Age±SD (years)
	Obese	Nonobese		
Sex				
Male				
Count	20	31	51	OM - 36.45±11.84
Percentage of group	55.6	72.1	64.6	OF - 40.09±7.51
Female				
Count	16	12	28	NOM - 36.45±11.23
Percentage of group	44.4	27.9	35.4	NOF - 31.45±10.74
Total				
Count	36	43	79	COM - 36.45±11.3
Percentage of group	100.0	100.0	100.0	COF - 36.43±10.79

OM: Obese male, OF: Obese female, NOM: Nonobese male, NOF: Nonobese female, COM: Combined male, COF: Combined female, SD: Standard deviation

HDL was significantly higher in obese females compared to obese males ( $P = 0.001$ ), and LDL was significantly higher in the obese group ( $P = 0.003$ ).

TNF- $\alpha$ , IL-6, and hs-CRP did not show any statistically significant difference in both groups and genders even though nonobese males and females showed higher values of IL-6 and obese and nonobese females showed higher values of hs-CRP, but none was statistically significant. AN was higher in females of both obese and nonobese groups ( $P = 0.002$ ). Obese males had lowered AN compared to nonobese males.

Table 4 was from our earlier study wherein we found no correlation of SCF and VF with IR and scattered correlations of components of metabolic syndrome with SCF and VF. Even none of the components of metabolic syndrome had any correlation with IR.

Table 5 shows the correlations of cytokines with SCF, VF, and IR in the obese group. Only TNF- $\alpha$  showed significant correlation with VF in obese males ( $P = 0.010$ ) and the rest IL-6, hs-CRP and AN had no significant correlation with SCF, VF, and IR both in males and females. TNF- $\alpha$  had significant correlation with both SCF and VF ( $P = 0.018$ ,  $P = 0.046$ ) in obese females, hs-CRP correlated significantly with VF ( $P = 0.009$ ) and VF/SCF ratio ( $P = 0.043$ ) and IL-6 had a significant correlation with IR ( $P = 0.043$ ) only in obese females.

In the nonobese group except for TNF- $\alpha$ , which had a significant correlation with IR in females ( $P = 0.021$ ), none of the other cytokines had any correlation with SCF, VF, and IR, shown in Table 6.

Table 7 shows the correlations of cytokines with the components of metabolic syndrome. hs-CRP had significant correlation with LDL in obese males ( $P = 0.042$ ) and with TC and LDL in obese females ( $P = 0.024$ ,  $P = 0.038$ ). IL-6 correlated with SBP and DBP in nonobese males ( $P = 0.016$ ,  $P = 0.034$ ) and TNF- $\alpha$  correlated with HbA1C in nonobese females ( $P = 0.030$ ). AN had no correlation with any of the components in both groups and genders.

## DISCUSSION

In this study, we have compared obese with nonobese nondiabetics. In our previous study, (MYVAD study) done in 2010,<sup>[20]</sup> we had compared obese and nonobese diabetics with obese and nonobese nondiabetics, wherein we found higher VF and higher IR in diabetics and obese nondiabetics but found no correlation of VF and SCF with IR. We observed in that study that there had to be some other reasons for the increased IR in our subjects. We conducted this study in the quest for finding whether chronic subclinical inflammation in the abdominal adipose tissue would be the reason for higher IR. Hence we estimated, TNF- $\alpha$ , IL-6,

**Table 3a: Physical, sonological, and biochemical findings of the participants of both groups**

Parameters	Obese		Nonobese		P	
	Male	Female	Male	Female	Group	Gender
BMI	28.30±3.14	31.75±4.99	21.21±1.65	19.67±2.14	0.000	NS
WC (cm)	101.75±6.53	94.81±6.80	84.70±5.36	73.58±4.94	0.000	0.000
SCF (cm)	1.32±0.46	2.00±0.54	0.96±0.34	1.00±0.42	0.000	0.001
VF (cm)	8.74±1.71	7.02±2.33	6.15±1.67	4.36±1.27	0.000	0.000
VF/SCF ratio	7.27±2.46	3.69±1.35	7.06±2.76	5.12±2.44	NS	0.000
SBP (mmHg)	127.2±11.56	130.0±13.63	123.29±13.21	120.0±14.14	0.023	NS
DBP (mmHg)	81.5±8.12	80.75±6.80	80.06±7.61	78.3±8.34	NS	NS
HbA1C (%)	5.67±0.44	5.87±0.42	5.56±0.45	5.47±0.34	0.014	NS
FBS (mg %)	92.8±6.73	94.0±7.20	92.51±8.33	88.58±9.33	NS	NS
FIL (mIU/ml)	14.65±10.42	9.92±7.28	9.56±5.45	9.47±6.87	NS	NS
IR	3.45±2.56	2.32±1.74	2.19±1.30	2.14±1.63	NS	NS

SCF: Subcutaneous fat, VF: Visceral fat, IR: Insulin resistance, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, NS: Not significant, BMI: Body mass index, WC: Waist circumference, FBS: Fasting blood sugar, FIL: Fasting insulin level, HbA1C: Hemoglobin A1c

**Table 3b: Lipid fractions, cytokines, and adipokine values of the participants in both groups**

Parameters	Obese		Nonobese		P	
	Male	Female	Male	Female	Group	Gender
TC (mg %)	171.2±24.84	183.87±27.27	165.00±65.00	157.00±25.85	0.028	NS
TG (mg %)	143.55±56.93	119.56±42.93	128.29±41.70	98.33±30.49	NS	0.01
HDL (mg %)	39.15±3.88	46.12±6.67	42.32±6.72	44.91±4.98	NS	0.001
LDL (mg %)	113.80±25.50	126.12±31.99	104.41±25.56	95.75±23.89	0.003	NS
TNF- $\alpha$ (pg/ml)	4.35±6.23	2.82±0.71	2.78±0.99	2.82±0.62	NS	NS
IL-6 (pg/ml)	4.16±2.32	4.52±2.21	5.61±5.28	6.72±6.58	NS	NS
hs-CRP (mg/dl)	3.32±3.83	4.48±4.08	2.50±3.62	3.96±5.00	NS	NS
Adiponectin (ng/ml)	10,536.98±6177.78	21,721.31±19,556.39	13,345.93±7459.20	18756.29±8363.92	NS	0.002

TC: Total cholesterol, TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, TNF- $\alpha$ : Tumor necrosis factor-alpha, IL-6: Interleukin-6, hs-CRP: High sensitive C-reactive protein, NS: Not significant

**Table 4: Pearson's correlation of BMI, WC, SCF, and VF with components metabolic syndrome (earlier study)<sup>[20]</sup>**

Parameters	SBP	DBP	HDL	TC	TG	LDL	IR
BMI	NS	0.047 (D)	NS	NS	NS	NS	NS
WC	NS	0.002 (B) 0.032 (D)	NS	NS	0.047 (B) 0.042 (C)	NS	NS
SCF	0.008 (A)	NS	NS	0.046 (D)	NS	0.048 (D)	NS
VF	NS	NS	NS	NS	0.004 (D)	NS	NS

SCF: Subcutaneous fat, VF: Visceral fat, IR: Insulin resistance, LDL: Low-density lipoprotein, TC: Total cholesterol, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, HDL: High-density lipoprotein, TG: Triglyceride, NS: Not significant, BMI: Body mass index, WC: Waist circumference

hs-CRP, which are proinflammatory cytokines and AN, which is an anti-inflammatory adipokine to know whether these would have any causal role for higher IR.

Our primary objective was to find out the levels of proinflammatory cytokines such as TNF- $\alpha$ , IL-6, and hs-CRP, in those having abdominal adiposity and IR and to know whether they would correlate with VF and SCF to establish a causal link for IR.

There is disagreement among many researchers about the portal theory for the causation of IR. There is a controversy as to whether VF or SCF is the culprit. Experiments showing adipose tissue derived proinflammatory cytokine-like

TNF- $\alpha$ , could actually cause IR in experimental models, provided the necessary impetus for further studies. This concept of VF as a site for the production of cytokines quickly extended beyond TNF- $\alpha$ , to include IL-6, hs-CRP, leptin, MCP-1, PAI-1, visfatin, etc. TNF- $\alpha$  and IL-6 and other cytokines appear to participate in the induction and maintenance of chronic sub-acute inflammatory state associated with obesity and activate cellular pathways that produce the development of IR.<sup>[1,5]</sup> Both TNF- $\alpha$  and IL-6 have been demonstrated to interfere with insulin signaling.<sup>[9]</sup> It has been shown that increase in VF levels was associated with increased levels of hs-CRP, TNF- $\alpha$ , HOMA-IR, oxidized LDL, etc.<sup>[6,13]</sup> IL-6 production in VF is 3-fold higher compared to SCF making abdominal adipose tissue a high-risk factor for the development of IR and hs-CRP which is an acute phase reactant produced by the liver in response to IL-6 has been correlated to IR.<sup>[7,10,13,16,25-27]</sup> No significant correlation of abdominal fat with IL-6 was also found in one of the studies.<sup>[28]</sup>

The IR as measured by the HOMA-IR values for normal individuals was 1.35–1.96.<sup>[23]</sup> It was 1.95 according to another study.<sup>[8]</sup> Both obese and nonobese males and females of this study showed higher values than that are mentioned above, which meant that all were insulin resistant. Obese males showing higher IR [Table 3a]. The

**Table 5: Pearson's correlation of cytokines, with abdominal adiposity, and IR (obese group)**

Parameters	OM				OF			
	TNF- $\alpha$	IL-6	hs-CRP	Adiponectin	TNF- $\alpha$	IL-6	hs-CRP	Adiponectin
SCF	NS	NS	NS	NS	0.018	NS	NS	NS
VF	0.010	NS	NS	NS	0.046	NS	0.009	NS
VF/SCF	NS	NS	NS	NS	NS	NS	0.030	NS
IR	NS	NS	NS	NS	NS	0.043	NS	NS

NS: Not significant, hs-CRP: High sensitive C-reactive protein, IL-6: Interleukin-6, TNF- $\alpha$ : Tumor necrosis factor-alpha, SCF: Subcutaneous fat, VF: Visceral fat, IR: Insulin resistance, OM: Obese male, OF: Obese female

**Table 6: Pearson's correlation of cytokines with abdominal adiposity and IR, (nonobese group)**

Parameters	NOM				NOF			
	TNF- $\alpha$	IL-6	hs-CRP	Adiponectin	TNF- $\alpha$	IL-6	hs-CRP	Adiponectin
SCF	NS	NS	NS	NS	NS	NS	NS	NS
VF	NS	NS	NS	NS	NS	NS	NS	NS
VF/SCF	NS	NS	NS	NS	NS	NS	NS	NS
IR	NS	NS	NS	NS	0.021	NS	NS	NS

NS: Not significant, hs-CRP: High sensitive C-reactive protein, IL-6: Interleukin-6, TNF- $\alpha$ : Tumor necrosis factor-alpha, SCF: Subcutaneous fat, VF: Visceral fat, IR: Insulin resistance, NOM: Nonobese male, NOF: Nonobese female

**Table 7: Pearson's correlation of cytokines with HbA1C, SBP, DBP, TC, TG, HDL, and LDL**

Cytokines	Obese		Nonobese	
	Male	Female	Male	Female
TNF- $\alpha$	NS	NS	NS	HbA1C - 0.030
IL-6	NS	NS	SBP - 0.016 DBP - 0.034	NS
hs-CRP	LDL - 0.042	TC - 0.024 LDL - 0.038	NS	NS
Adiponectin	NS	NS	NS	NS

TNF- $\alpha$ : Tumor necrosis factor-alpha, NS: Not significant, LDL: Low-density lipoprotein, TC: Total cholesterol, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, HDL: High-density lipoprotein, hs-CRP: High sensitive C-reactive protein, IL-6: Interleukin-6, HbA1C: Hemoglobin A1c

normal values for the cytokines were TNF- $\alpha$  (0–5.9 pg/ml), IL-6 (<4 pg/ml), and hs-CRP (0–0.05 mg/dl). Males and females of both groups of this study showed higher than the normal values for IL-6, nonobese having higher values than the obese, which was contrary to the assumption that obese people should have had higher IL-6 than the nonobese. The values of TNF- $\alpha$  were within the normal limits even though obese males had higher values compared to others. hs-CRP values were significantly higher in the participants of both groups, females in both groups had higher values compared to males. However, none reached the level of statistical significance [Tables 3b and 5].

TNF- $\alpha$  correlated with VF in both obese males and females, IL-6 did not correlate both with VF and SCF and hs-CRP correlated with only with VF in obese females. None of the cytokines correlated with either SCF or VF in the nonobese males or females. IL-6 correlated with IR in obese females and TNF- $\alpha$  correlated with IR in nonobese females and hs-CRP did not correlate with IR in both groups. TNF- $\alpha$ , which correlated with VF in obese

had no correlation with IR, whereas it correlated with IR in nonobese females but had no correlation with their SCF or VF. IL-6, despite no correlation with either SCF or VF, correlated with IR in obese females. hs-CRP correlated with VF in obese females but had no correlation with their IR. No linear correlation of increased VF or SCF with increased proinflammatory cytokines could be established in this study. Hence, no causal link could be established between the cytokines, abdominal adiposity, and IR.

The secondary objective was to know whether these cytokines vary in those who have had IR compared to those without IR, irrespective of whether they are metabolically obese or not.

Since both obese and nonobese males and females showed higher IR values than the normal, there was no group without IR. Nonobese males and females despite having lower VF showed IR; whereas obese males and females had higher VF and higher IR. IL-6 and hs-CRP values were higher in nonobese males and females where IR was lower compared to others. TNF- $\alpha$  was higher in obese males with higher VF and higher IR even though it did not correlate with IR in them. There was no linear relationship between increase in cytokines and increased IR, neither vice versa.

The other objective was to ascertain the significance of AN in the causation of IR.

AN is produced by adipocytes, is anti-inflammatory, and its expression decreases with increased adiposity.<sup>[1]</sup> Hypoadiponectinemia is common in obese adults and in subjects having IR. Low AN values were shown to be predictive of future diabetes in many populations including

Asian Indians.<sup>[17]</sup> AN levels were significantly lower (by 2-fold) in the insulin resistant than in insulin sensitive subjects and was inversely correlated with IR.<sup>[18,29]</sup> Insulin sensitivity rather than obesity or percentage of body fat was the major determinant of AN levels.<sup>[29]</sup> In Asian Indian adults and teenagers, AN did not correlate directly with the measurement of insulin sensitivity and overweight.<sup>[9]</sup> Decreased AN levels in obese women were associated with higher levels of hs-CRP and IL-6 and lead to IR and hyperinsulinemia.<sup>[24]</sup>

This study showed higher than normal values of AN, in women of both groups compared to men ( $P = 0.002$ ). None of the values were below the normal (3000–14,000 ng/ml). AN did not correlate with SCF, VF, and IR in both obese and nonobese males and females. All that we could say from this study was that AN was higher in females, but its contribution toward IR could not be established.

As far as components of metabolic syndrome and their relationship with cytokines, studies have shown AN correlating positively with HDL and negatively with TG<sup>[25]</sup> and with SBP and DBP.<sup>[29]</sup> Low HDL levels were considered to be consistent with low AN values in Asians.<sup>[17]</sup>

This study showed IL-6 correlating positively with SBP and DBP in nonobese males, hs-CRP correlating with LDL in both obese males and females, TNF- $\alpha$  correlating with HbA1C in nonobese females and AN not correlating with any parameter in both groups [Table 7]. No conclusion could be drawn with such a scattered correlation, suffice to say cytokines in this study may not be responsible for the changes in the metabolic parameters.

This study has shown higher VF and higher IR in males, whether they were obese or not, higher IL-6 and hs-CRP values in all the participants, higher AN in females, a significant correlation of TNF- $\alpha$  with VF both in obese males and females, despite normal values. However, no consistent correlation of cytokines with VF, SCF, and IR.

The limitation of the study is the size. An increased number of subjects, probably would have given more meaningful results, but the cost was the constraint. Even with the number of subjects involved in this study, we expected a causal link or at least a trend, between cytokines, VF, and IR, as shown in many of the studies referred in this study, but unfortunately could not be demonstrated.

## CONCLUSIONS

In this study on South Indian subjects, proinflammatory cytokines like IL-6 and hs-CRP, despite being high, did not

show any causal correlation either with abdominal obesity or with IR. TNF- $\alpha$  being normal showed some correlation which was inconsistent. Even the anti-inflammatory adipokine, AN did not show any correlation with IR. Cytokines were found to have inconsistent correlations with the components of metabolic syndrome hence were not useful.

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## Conflicts of interest

There are no conflicts of interest.

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