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Original Research Article

Association of obesity with C reactive protein, total leukocyte count and erythrocyte sedimentation rate in Type 2 diabetes mellitus

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Abstract

Context: There is accumulating evidence implicating inflammation originating from adipose tissue as a potential pathway in the pathogenesis of type 2 diabetes mellitus (DM). This study is performed to ascertain the association of 3 markers of inflammation, leukocyte count (TLC), Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) with obesity in diabetics and compare with non-diabetics.

Aims: To determine the association of CRP, TLC and ESR levels with obesity in persons with type 2 DM and non-diabetics.

Settings and Design: 50 diabetics and 50 non-diabetic subjects were enrolled prospectively and anthropometric and laboratory data were collected and analysed in a tertiary care hospital.

Methods and Material: CRP by latex agglutination test, TLC by Cell counter method (BC-5380), ESR by Westerngren's method.

Statistical analysis: Pearson's correlation, Chi square and Fisher's exact test -Mean, Standard deviation, Frequency and Percentage.

Results: Mean FBS, PPBS, ESR, CRP, TLC, Body mass index (BMI), Waist hip ratio (WHR) were 100.5mg/dl, 112.34mg/dl, 7.02mm/hr, 1.86mg/L,6984cells/mcL,24.77kg/m^2,0.88 respectively in non-diabetics and 190.88mg/dL, 275.58mg/dL, 21.56mm/hr, 3.91mg/L, 11760 cells/mcL, 25.86kg/m^2, 0.98 respectively in diabetics. All these parameters except BMI are significantly different between diabetics and non-diabetics with p value <0.05. Pearson correlation showed statistically significant association of inflammatory markers with WHR but not with BMI.

Conclusions: Inflammatory markers like TLC, ESR and CRP are significantly elevated in patients with diabetes, suggesting the important role of inflammation, prompting need for interventions reducing inflammation in effective diabetes management. WHR is significantly associated with elevated inflammatory markers suggesting the role of abdominal obesity in diabetes.

Keywords: Type 2 diabetes, inflammatory markers, BMI, Waist hip ratio.

1. Introduction

Type 2 diabetes mellitus (DM) is a major growing public health problem. There is an urgent need to develop primary prevention strategies aimed at controlling this epidemic. [1]

It has recently been shown that patients with diabetes tend to have a higher body mass index (BMI) and there is accumulating evidence implicating inflammation originating from adipose tissue as a potential pathway in the pathogenesis of type 2 DM. Obesity is a proinflammatory state and inflammatory mechanisms interfere with insulin signal transduction. Obesity can cause low-grade chronic inflammation through enhanced adipose tissue-derived cytokine expression, and inflammatory factors are thus

likely in the pathway that links obesity to insulin resistance. [1-3] Substantial experimental evidence and more recent cross sectional data suggests that interleukin 6 (IL-6) and C-reactive protein (CRP), 2 sensitive physiological markers of sub-clinical systemic inflammation, are associated with hyperglycemia, insulin resistance and overt type 2 DM. [4]

IL-6 and TNF-α are released in significant amounts from adipose tissue, particularly visceral adipose tissue. Their release from adipose tissue is augmented by increased sympathetic stimulation which is down-regulated by regular physical activity. TNF- α is a potent stimulator of IL-6 production and IL-6 is a potent stimulator of fibrinogen, WBC and CRP. Elevations in TNF and IL-6 are associated with insulin resistance and dyslipidemia. [5,6]

CRP an acute-phase reactant is a marker of inflammation produced primarily in the liver. CRP is widely available, stable and has standardized assays for its measurement. It is strongly associated with BMI and waist circumference. [7,8]

Of the markers of inflammation, CRP has been shown in multiple prospective studies to predict incident myocardial infarction, stroke, peripheral vascular disease, and sudden cardiac death. [5]

Very few comparison studies have been performed so far. Thus to further elucidate the mechanisms involved in the pathogenesis of type 2 DM, the present study is taken up to examine the association between CRP, ESR and leukocyte count with measures of obesity in diabetics and non diabetics.[5]

Lifestyle changes are the first-line treatment in the prevention of diabetes. Weight loss combined with physical activity and dietary changes have been shown to decrease markers of inflammation in obese individuals.

2. Material and Methods

The purpose of this study is to determine the association of CRP, Total Leukocyte count (TLC) and ESR levels with obesity in persons with type 2 DM and non diabetic individuals. This is a prospective case control study conducted in a tertiary care hospital in Mangalore, after the approval from institutional ethical committee.

2.1 Study group:

50 Known cases of Type 2 DM of either sex were included by random sampling method in the study after an informed and written consent duly signed by each participant.

2.2 Control group:

50 non diabetic subjects of either sex were included by random sampling method in the study after an informed and written consent duly signed by each participant.

2.3 Inclusion criteria

50 cases of diagnosed type-2 DM, confirmed by biochemical investigations as per WHO criteria.

2.4 Exclusion criteria

Patients with other chronic inflammatory conditions causing rise in the level of CRP, TLC and ESR. A detailed history as per a preformed case proforma was taken noting patient's past history about the patient's DM, its complications and its management from both outpatient and Inpatient department followed by a detailed systemic examination.

2.5 Anthropometric measurements

Height, weight, and waist and hip circumferences were taken as per the WHO STEPS protocol. The WHO STEPS protocol recommends that the subject stands with arms at the sides, feet positioned close together, and weight evenly distributed across the feet and should wear little clothing. The subject should be relaxed, and the measurements should be taken at the end of a normal expiration. Waist circumference is to be measured at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest. The Hip circumference measurement should be taken around the widest portion of the buttocks. BMI is the ratio between weight in kilograms and the square of height in meters. Waist hip ratio (WHR) was calculated as waist (cm)/hip (cm).

The established guidelines for BMI in Indians are

Underweight (<18.5 kg/m^2)
Normal (18.5 - 22.9 kg/m^2)
Overweight (23-24.9 kg/m^2)
Obese (>25 kg/m^2).

The WHO defines the normal Waist to hip ratios of <0.9 in men and <0.8 in women.

2.6 Statistical analysis

-Pearson's correlation, Chi square test and Fisher's exact test

-Mean, Standard deviation, Frequency and Percentage

Under aseptic precautions, 2ml of plain blood is drawn by venepuncture CRP was measured by latex agglutination test. The principle of the test is as follows, uniform latex particles are coated with anti-human CRP. The specimen containing CRP on mixing with latex reagent agglutination, showing positive test result. If CRP is absent, there will be no agglutination, indicating a negative result. Concentration of CRP was calculated using,

CRP (mg/dl) =S×D

Where, S=Sensitivity of the reagent i.e. 0.6 mg/dl

D=Highest dilution of serum showing agglutination.

TLC by Cell counter method (BC-5380).

ESR by Westerngren's method.

3. Results

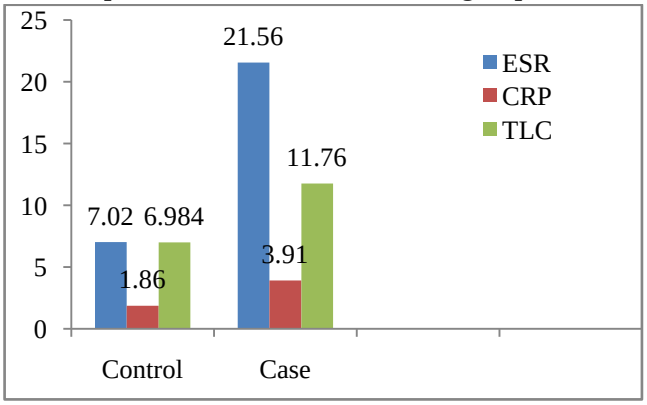
It is a prospective case control study in which 100 subjects were included. Out of which 50 (30 males and 20 females) were non-diabetic apparently healthy control subjects and 50 (29 males and 21 females) were Type 2 DM patients confirmed by biochemical investigations as per WHO criteria. (Table 1) The age group of cases and controls were between 20-80 years with a mean age of 46 for controls and 53 for cases (Table 1).

Table 1: Comparison of Gender and Age Parameters in Control and Case Groups

Parameters	Groups	
Gender	Control(n=50)	Male = 30 Female = 20
	Case(n=50)	Male = 29 Female = 21
Age in years (Mean)	Control	46
	Case	53

FBS, PPBS, TLC, ESR, CRP levels was measured in these subjects. Table 2 shows routine biochemical parameters such as fasting, post prandial blood sugars, ESR, CRP and TLC done in all subjects. They were significantly higher in cases than in controls. The mean value of FBS in control group was found to be 100.50mg/dL and in case group it was 190.88mg/dL. The mean value of PPBS in control group was found to be 112.34mg/dL and in case group it was found to be 275.58mg/dL. The mean value of ESR in control group was found to be 7.02mm/hour and in case group it was 21.56mm/hour. The mean value of CRP in control group was found to be 1.86mg/L and in case group it was 3.91mg/L. The mean value of TLC in control group was found to be 6984 cells/ mcL and in case group it was 11760 cells/ mcL (Figure 1).

Figure 1: Comparison of FBS, PPBS, ESR, CRP, TLC parameters in control and case groups



Independent sample T-test: Table 2 shows that the p values are <0.05 between cases and controls for FBS, PPBS, ESR, CRP, TLC. Hence all selected characteristics are different between diabetics and non diabetics at 5% level of significance.

Table 2: Comparison of FBS, PPBS, ESR, CRP, TLC Parameters in Control and Case Groups

Parameters		Mean	Std Deviation	p Value
Fasting Blood Glucose (FBS) (mg/dL)	Control	100.98	11.52	<0.001
	Case	134.80	45.96	
Post Prandial Blood Glucose (PPBS) (mg/dL)	Control	108.76	25.27	<0.001
	Case	236.6	88.10	
ESR (mm/hour)	Control	7.02	3.59	<0.001
	Case	21.56	17.31	
CRP (mg/L)	Control	1.86	0.67	<0.001
	Case	3.91	0.87	
TLC (cells/ mcL)	Control	6984	1441.98	<0.001
	Case	11760	4458.97	

BMI and waist to hip circumference was measured in these subjects. Table 3 shows that the mean value of BMI in control group was found to be 24.77 kg/m^2 and in case group it was 25.86 kg/m^2. The mean value of WHR in control group was found to be 0.88 and in case group it was 0.98. Independent sample T-test: Table 3 shows that the p values are <0.05 for WHR but not for BMI (0.168). Hence except BMI the selected characteristics are different between diabetics and non diabetics at 5% level of significance.

Table 3: Comparison of BMI, Waist to Hip Ratio Parameters in Control and Case Groups:

Parameters		Mean	Std Deviation	p Value
BMI (kg/m^2)	Control	24.77	3.02	0.168
	Case	25.86	4.67	
Waist to hip circumference ratio	Control	0.88	0.073	<0.001
	Case	0.98	0.116	

Pearson's correlation coefficient was used to find out the association between ESR, TLC, CRP and BMI and also the association between ESR, TLC, CRP and WHR. Table 4 shows that the p values are >0.05 hence there is no association between BMI and other parameters at 5 % level of significance.

Table 4: Pearson's Correlation between ESR, TLC, CRP and BMI

Pearson Correlation	r Value	p Value
ESR v/s BMI	0.082	0.417
TLC v/s BMI	0.184	0.066
CRP v/s BMI	0.097	0.336

Table 5 shows that the p values are <0.05 hence there is association between WHR and other parameters at 5 % level of significance.

Table 5: Pearson's Correlation between ESR, TLC, CRP and Waist to Hip Ratio

Pearson Correlation	r Value	p Value
ESR v/s Waist to hip ratio	0.338	0.001
TLC v/s Waist to hip ratio	0.306	0.002
CRP v/s Waist to hip ratio	0.407	<0.001

4. Discussion

Diabetes accounts for a significant part of the morbidity and mortality. Diabetes is estimated to affect 25.6 million American adults and 366 million people worldwide, and the numbers will continue to increase to 552 million by 2030 globally. Type 2 diabetes makes up >90% of all diabetes cases. Therefore, primary prevention of type 2 diabetes through diet and lifestyle modifications is of paramount public health importance.

Subclinical inflammation and presence of almost all indicators of systemic inflammation are found in type 2 diabetic patients. The rise in the inflammatory cytokines is the essential step in glucotoxicity and lipotoxicity induced mitochondrial injury, oxidative stress and beta cell apoptosis in type 2 DM [12].

In our cross sectional study of 100 subjects, 50 diabetics (29 males and 21 females) and 50 non diabetics (30 males and 20 females) of 20-80 age groups were included. The mean age of 46 years for controls and 53years for case group was observed. Routine biochemical parameters such as FBS and PPBS were done in all subjects which were significantly higher in cases than in controls.

FBS in control group was found to be 100.50 mg/dL and in the case group it was found to be 190.88mg/dL. This was in concordance when compared to the study done by Shrabani Mohanty, *et al* (2013) which showed that FBS in their control group was 81.96mg/dL and 218.62mg/dL in case group.

PPBS value in control group of our study was found to be 112.34 mg/dL and in case group it was 275.58 mg/dL. This was in concordance with their study which showed 113.56mg/dL for control group and 285.04mg/dL for case group. [13]

In our study the mean value of ESR in control group was found to be 7.02 mm/hour and in case group it was 21.56mm/hour. The mean value of CRP in control group was found to be 1.86mg/L and in case group it was 3.91mg/L. The mean value of TLC in control group was found to be 6984 cells/ mcL and in case group it was 11760 cells/ mcL. The mean value of BMI in control group was found to be 24.77kg/m^2 and in case group it was 25.86 kg/m^2. The mean value of WHR in control group was found to be 0.88 and in case group it was 0.98.

Similar such increase in ESR, CRP, TLC, BMI and WHR levels in diabetic patients as compared to controls has been reported by some authors.[14-18] (Table 6).

Table 6: Comparison between ESR, CRP, TLC, BMI, WHR in Various Studies with Our Study

Studies	Group	ESR	CRP	TLC	BMI	WHR
Ghanem *et al* [11]	Control		0.214		22.8±1.0	
	Case		0.243		32.3±2.1	
Hu *et al* [12]	Control		0.16		26.2 ± 6.1	
	Case		0.36		30.3 ± 5.6	
Spranger *et al* [13]	Control		2.45 ± 4.38		26.7 ± 3.5	0.89 ± 0.09
	Case		4.14 ± 5.1		30.7 ± 4.8	0.95 ± 0.09
Duncan *et al* [14]	Control			5500 (4600–6600)	26.1 (23.7–28.9)	0.91 (0.85–0.96)
	Case			6100 (5100–7300)	30.0 (26.7–33.9)	0.96 (0.92–1.00)
Pradhan *et al* [15]	Control		0.26		25.6	
	Case		0.69		31.8	
Our study	**Control**	**7.02**	**1.86**	**6984**	**24.77**	**0.88**
	Case	**21.56**	**3.91**	**11760**	**25.86**	**0.98**

In a study done by Amina Nadeem, *et al* (2013) showed that ESR, TLC, IL-6 and TNF-α were found to be significantly higher in T2DM patients. There was also statistically significant difference in BMI, fasting blood sugar, insulin resistance and dyslipidemia (except LDL) between diabetic and control group. All inflammatory markers except TNF-α were positively correlated with insulin resistance. Only TNF-α was found to be correlated to duration of the disease. Both TLC and Serum insulin were positively correlated to BMI. Inflammatory markers were not correlated to dyslipidemia. [12]

Adipocytes secrete inflammatory cytokine. In addition they develop macrophage infiltration (adiposities) which is the source of almost all TNF–α and most of the IL-6 in adipose tissue along with other inflammatory markers. Not only obesity but hyperinsulinism per se, seen in

metabolic syndrome and type 2 DM, can induce rise in inflammatory markers including IL-6, TNF-α and CRP3. High levels of IL-6 and TNF-α have been reported to be associated with insulin resistance in adipocytes, hepatocytes and myocytes. Raised serum IL-6 and TNF-α level in type 2 diabetic patients were found to be associated with increased BMI, fasting insulin levels and insulin resistance. In a prospective study in American females, the baseline CRP and IL-6 levels were significantly higher in those who later developed T2DM. [12]

In another study done by Xia Wang, *et al* (2013) detected a significant dose-response association of IL-6 levels with type 2 diabetes risk (relative risk [RR] 1.31 [95% CI 1.17–1.46]). For CRP, the meta-analysis involving 22 cohorts, with a total of 40,735 participants and 5,753 cases, showed that elevated CRP levels were significantly associated with increased risk of type 2 diabetes (1.26 [1.16–1.37]), with the absence of publication bias. Sensitivity and subgroup analysis further supported the associations. [16]

In another study done by Ahmed Al-Shukaili, *et al* (2013), showed that BMI was positively correlated with CRP, TNF- α, and phosphate levels; and the weight was positively correlated with CRP and IFN- α, which is in accordance with previous findings. This correlation can be explained as follows: obesity is associated with enlargement of adipose tissue and consequently increases the number of adipose tissues macrophages. These macrophages are responsible for almost all adipose tissue TNF- α expressions and other acute-phase response markers and mediators of inflammation. TNF- α, secreted by adipose tissue, may play a critical role in insulin resistance and the pathogenesis of type 2 diabetes. Several studies indicated that increased levels of cytokines and acute-phase proteins can participate in maintaining the insulin-resistant state. [17]

In a study done by Frank B. Hu, *et al* (2004) among 32,826 women who developed diabetes during follow-up had significantly higher levels of BMI, waist circumference, and waist-to-hip ratio; had lower levels of physical activity and intake of cereal fiber; and were more likely to have a family history of diabetes than control subjects. BMI (kg/m2) was 30.3 in case and 26.2 in control. And CRP (mg/dl) was found to be 0.36 in case and 0.16 in control. [18]

In another study done by Srikanthan, *et al* (2009) found that there was no association between all-cause mortality and BMI or waist circumference in either unadjusted or adjusted analysis. In contrast, all-cause mortality increased with WHR. There was an interaction with sex, so that there was a graded relationship between WHR and mortality in women (relative hazard, 1.28 per 0.1 increase in WHR; 95% confidence interval, 1.05–1.55) and a threshold relationship in men (relative hazard 1.75 for WHR > 1.0 compared to WHR ≤ 1.0; 95% confidence interval, 1.06–2.91). [19]

4.1 Limitations of the study

Present study is a hospital based study and may not be a true representation of the population at large. Even though the present study has included the easily measurable cost effective markers of inflammation, levels of cytokines were not analysed. A follow up study of change in the inflammatory markers after reducing obesity and its impact on diabetic control would be more ideal.

5. Conclusions

Inflammatory markers like TLC, ESR and CRP are significantly elevated in patients with diabetes, suggesting the important role of inflammation, prompting need for interventions reducing inflammation in effective diabetes management. WHR is significantly associated with elevated inflammatory markers suggesting the role of abdominal obesity in diabetes.

Ethical issues: Nil

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