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Original Article

# Comparison of Inflammatory Markers like C - Reactive Protein, Total Leukocyte Count and Erythrocyte Sedimentation Rate in Type II Diabetes Mellitus

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Abstract :

Background: Diabetes Mellitus (DM) has become an epidemic in the 21st century where India leads the world with largest number of patients. There is increasing evidence that inflammation is closely involved in the pathogenesis of type 2 diabetes and associated complications such as dyslipidaemia and atherosclerosis. Many previous studies indicate inflammatory markers like CRP, IL-6, IL-8, TNF-alpha, fibrinogen, total sialic acid, ceruloplasmin and total leucocyte count (TLC) are raised in Type II Diabetes Mellitus. However, not many studies have done association of ESR and TLC in DM.

Aims: In the present study inflammatory markers like CRP, total leukocyte count and ESR were compared in diabetic and non-diabetic patients.

Materials and Methods: 5mL of venous blood was taken from the study subjects.CRP, TLC and ESR was estimated.

Results: There was a significant rise in the CRP, TLC and ESR values seen in patients diagnosed with type II diabetes mellitus when compared to normal individuals. All three parameters (CRP, TLC, and ESR) were raised in a total of 7 cases (14%). 14 cases (28%) showed elevated levels of both CRP and ESR. A significant 8 cases (16%) showed elevation of CRP alone. There was a rise of ESR alone in 5 cases (10%).

Conclusion: CRP, TLC and ESR are elevated in diabetic patients in comparison to normal individuals. These increase the risk of diabetic related complications like atherosclerosis and dyslipaedemia. Hence, anti-inflammatory drugs in combination with antidiabetic treatment can delay these complications.

## Introduction

Diabetes Mellitus is a metabolic disorder characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.[1] Type II Diabetes Mellitus (DM2) is the most common form of diabetes. The 'top' three countries in terms of the number of DM2 individuals with diabetes are India (31.7 million in 2000; 79.4 million in 2030), China (20.8 million in 2000; 42.3 million in 2030); and USA (17.7 million in 2000; 30.3 million in 2030).[2] Clearly, DM2 has become an epidemic in the 21st century where India leads the world with largest number of diabetic subjects. The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025.[3] DM2 results from the interaction between a genetic predisposition, behavioural and environmental risk factors.[4] The two metabolic defects that characterize DM2 are, [1]decreased ability of peripheral tissues to respond to insulin (insulin resistance) and [2]beta cell dysfunction that is manifested as inadequate insulin secretion in the face of insulin resistance and hyperglycaemia.[5] This form of diabetes is most often associated with older age, obesity, family history, previous history of gestational diabetes, physical inactivity and certain ethnicities. About 80% of

people with DM2 are overweight.[6] Diabetes is associated with long-term complications that affect almost every organ of the body. The disease often leads to blindness, heart, blood vessel disease, stroke, kidney failures, amputations and nerve damage. Uncontrolled diabetes can complicate pregnancy and birth defects are more common in babies born to women with diabetes.[6, 7, 8] However, there has been a significant increase in cases of DM2 under the age of 30 years. This is a great concern as future generations may be burdened with morbidity and mortality at the cost of their productivity, potentially affecting the workforce and healthcare resources of the countries across the world.[6, 9, and 10]

Wang et al., in 2012 conducted a complete meta-analysis on the role of inflammatory markers in DM2. They concluded that C-Reactive Protein (CRP) and Interleukin-6 had significant association with Diabetes.[7] However, not many studies have been carried out in world literature to ascertain the role of Total Leucocyte Count (TLC) and Erythrocyte Sedimentation Rate (ESR) in DM2. In the present proposed study our primary attempt is to make a comparative study of the effect of DM2 on various serum inflammatory markers like C - reactive protein (CRP), Total Leucocyte Count (TLC) and Erythrocyte Sedimentation Rate (ESR). The results of this study can be of great prognostic value.

## Materials and methods

The study was approved by the Institutional ethics committee (INST.EC/EC/51/2016-17). This prospective study was conducted in a tertiary hospital of coastal Karnataka. Total of 50 Diabetes and 50 Healthy control subjects were included in the study. The criteria for Control Group include non-Diabetic individuals from general population more than 20 years and who are willing to give the written consent. The criteria for diabetic group include patients diagnosed with DM2 based on international protocol with age group more than 20 years and who are willing to give the written consent. We excluded smokers, alcoholics, hypertensives, obese individuls, previous organ transplants, patients with infections / tissue injury/ recent surgeries/ autoimmune disorders/ ischemic heart disease or malignancy.

Participants were carefully selected based on set inclusion and exclusion criteria. Written Informed Consent was also taken from the subject having explained all details regarding the study the in language understood to them.

5mL of venous blood were taken from the participants and collected in two different vacutainers i.e. Plain and EDTA-treated vacutainers. The plain blood sample was centrifuged at 2500rpm for 10 minutes; serum was separated and used for estimation of CRP (quantitative turbidometric method). Blood collected in the EDTA-treated vacutainer was used to determine Total Leukocyte Count (TLC) and Erythrocyte Sedimentation Rate. TLC was estimated using autoanalyser (BC-5360), whereas ESR was calculated manually using westergren's pipettes.

## Statistical Analysis

The collected information was summarised by using the descriptive statistics such as frequency, percentage, minimum, maximum, mean, standard deviation, median and IQR (Inter-Quartile Range). To compare the TLC (c/cu.mm)(Table 1) , ESR (mm/hr) (Table 2), CRP (mg/l)(Table 3) between case and control Mann-Whitney U test (Inferential statistics) was used. The p value <0.05 was considered significant.

## Results

A total no. of 100 individuals (50 cases and 50 controls) was systemically identified using the prescribed inclusion and exclusion criteria. There were 70 males and 30 females; varying from age groups from 20 to 80 years. Mean age was 45 years.

The following observations were made among the 50 cases. There was a significant rise in the CRP, TLC and ESR values seen in patients diagnosed with type II diabetes mellitus when compared to normal individuals. All three parameters (CRP, TLC, and ESR) were raised in a total of 7 cases (14%). 14 cases (28%) showed elevated levels of both CRP and ESR. 1 case (2%) showed elevated CRP and TLC levels. There were no cases showing increase in TLC with

ESR. A significant 8 cases (16%) showed elevation of CRP alone. There was a rise of ESR alone in 5 cases (10%). No cases were seen with increased TLC alone. Only 15 participants (30%) showed normal CRP, ESR and TLC values. The diagramatic representation of the results are shown below in Fig.1

Table 1: Descriptive Statistics and Mann Whitney U test for Total Leucocyte count between case and control subjects. P<0.05 is considered statistically significant.

|  | Maximum | Minimum | Mean | Standard Deviation | Median | IQR | Mann-Whitney U | P value |
|---|---|---|---|---|---|---|---|---|
| Case | 16100 | 4800 | 8586 | 2557.822 | 8350 | 6950-9500 | 659.5 (Z=4.072) | <0.001 |
| Control | 11400 | 3800 | 6690 | 1568.471 | 6800 | 5450-7425 | | |

Table 2 : Descriptive Statistics and Mann Whitney U test for Erythrocyte Sedimentation Rate between case and control subjects. P<0.05 is considered statistically significant.

|  | Maximum | Minimum | Mean | Standard Deviation | Median | IQR | Mann-Whitney U | P value |
|---|---|---|---|---|---|---|---|---|
| Case | 116 | 3 | 31.98 | 34.731 | 17.50 | 5-50 | 585 (Z=4.628) | <0.001 |
| Control | 30 | 3 | 7.18 | 6.110 | 5 | 3-8.5 | | |

Table 3: Descriptive Statistics and Mann Whitney U test for C - reactive protein between case and control subjects. P<0.05 is considered statistically significant.

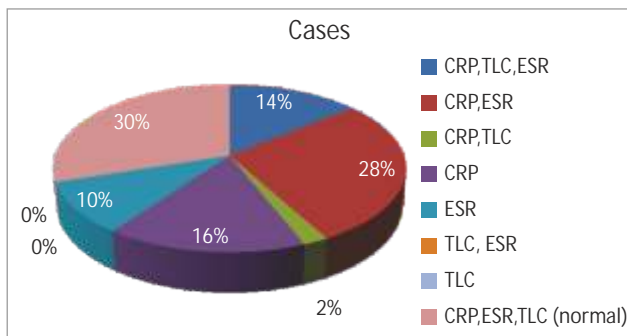|  | Maximum | Minimum | Mean | Standard Deviation | Median | IQR | Mann-Whitney U | P value |
|---|---|---|---|---|---|---|---|---|
| Case | 292.6 | 0.5 | 42.920 | 70.0211 | 8.680 | 1.768-54.412 | 461 (Z=5.443) | <0.001 |
| Control | 10.2 | 0.5 | 1.877 | 2.0411 | 1.360 | 0.688-1.978 | | |



Fig 1 : Pie-chart representing increase in inflammatory markers in cases

## Discussion

Type II Diabetes Mellitus is a global health problem in the 21$^{st}$ century. Inflammation is a key regulator in the pathogenesis of complications associated with Diabetes. Hence, assessment of inflammatory markers might help to follow up all diabetic patients and assess the severity of their complications. Many previous studies indicate inflammatory markers like CRP, IL-6, IL-8, TNF-a, fibrinogen, total sialic acid, ceruloplasmin and TLC are raised in Type II Diabetes Mellitus.[7, 8] However, and not many studies have been done on T2DM with the association of Erythrocyte Sedimentation Rate and Total Leucocyte Count.

In our present study, we have compared the levels of C - reactive protein, Total Leucocyte Count and Erythrocyte Sedimentation Rate with Type II Diabetes Mellitus.

Mugabo Y et al. in the year 2010 demonstrated the role of CRP in the development of diabetic vasculopathy.[11] Ridker P in the year 2003 concluded that CRP is also an independent indicator of future cardiovascular events that adds prognostic information to lipid screening, metabolic syndrome and Framingham Risk Score.[12] Our studies showed high levels of CRP in 60% of the Diabetic patients. The high levels of CRP demonstrated in our study can indicate future cardiovascular diseases in these individuals.

Elias AN and Domurat E in the year 1989 had conducted a study to demonstrate the relationship between ESR and diabetes.[13] they reported an increase in ESR levels among diabetics. Our present study also shows an increase in ESR levels in 50% of the test subjects. ESR can be used as an important marker for diagnosis of T2DM. ESR    100 mm/hr can indicate hidden malignancies which were not diagnosed previously. In our study, 6 cases showed ESR more than 100. However, these patients were not suffering from any malignancies.

Fu-Mei Chung et al. in the year 2005 demonstrated the role of leukocytes in the development and progression of

diabetic neuropathy.[14] Raised TLC can help in diagnosis of any hidden infections in such diabetic patients. In our study, 16% cases showed increase in TLC levels. These patients were treated with empirical antibiotics. Unfortunately, the long term follow up of these patients could not be done.

## Conclusion

After comparing all three inflammatory markers; C reactive protein, Total leukocyte count and Erythrocyte sedimentation rate in patients of Type II Diabetes Mellitus, we found that all the three parameters are increased in these patients in comparison to normal individuals. Among the three, CRP and ESR are significantly elevated inT2DM. These may increase the risk of diabetic related complications like atherosclerosis and dyslipaedemia. Hence, anti-inflammatory drugs in combination with antidiabetic treatment can delay these complications.

## Acknowledgement

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