TO STUDY THE SERUM LEVELS OF HSCRP IN PRE-DIABETES MELLITUS PATIENTS & TO COMPARE THAT OF NORMAL SUBJECTS

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ABSTRACT

BACKGROUND
Diabetes Complications is one of the major long-term complications of diabetes mellitus. Previous studies have proved that Type 2 Diabetes is frequently associated with an inflammatory status with increased levels of serum hsCRP, an inflammatory marker, but little information is available on the relationship between low-grade inflammation and diabetic early complications.

OBJECTIVES
1. To determine the serum level of hsCRP in Type-2 Pre diabetics patients with and without early diabetic complications and to compare with that of normal subjects. 2. To study the association of serum hsCRP levels with glycosylated haemoglobin and AC ratio.

METHODOLOGY
56 Type 2 Pre Diabetic patients in the age range of 50-60 years with >10years of duration of diabetes were recruited. Diabetic patients were divided into 2 groups, diabetic patients with AC ratio between 30-300 mg/gm and diabetic patients with AC ratio >300mg/gm. 24age and sex matched normal subjects were recruited for this study. Serum hsCRP levels were measured by turbidimetry method.

RESULTS
There was a significant increase in serum hsCRP levels in both the diabetic groups (mean=4.77±3.95) compared to normal controls (mean=2.35±2.64). The hsCRP levels showed a positive correlation with HbA1c (r =0.38, p=0.007) and AC ratio (r= 0.33, p=0.01) in diabetic patients.

CONCLUSION
Serum hsCRP is increased in prediabetes patients and correlates with HbA1c and albuminuria. Thus, estimation of serum hsCRP levels helps in early intervention and prevention of further complications in diabetic prediabetes patients.

KEYWORDS
Pre-diabetes, AC Ratio, Inflammation, hsCRP, HbA1c.

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INTRODUCTION: Previous studies have proved that Type 2 PRE DIABETICS is frequently associated with chronic inflammatory state.1,2 Chronic inflammation plays an important role in the development of diabetes and its late complications.3,4 C-reactive protein (CRP) an acute phase reactant is a highly sensitive marker of inflammation. Its levels rise dramatically during an inflammatory processes.5 As CRP has a long half-life, affordability of estimation, stability of levels with a circadian variation is best among the markers of vascular inflammation.6 CRP has been found to be associated with disorders like diabetes mellitus, cardiovascular disorders, metabolic syndrome, renal failure etc.2,7,8 Serum hsCRP level is higher in patients with Type 2 diabetes than in normal subjects and plays an important role in the development and progression of Type 2 diabetes mellitus.9 It is also shown that this marker of inflammation correlate with the measures of glycemic control, such as glycosylated haemoglobin A1c (HbA1c).10 But little information is available on the relationships between low-grade inflammation and prediabetes patients. Therefore, the present study was undertaken to evaluate Serum hsCRP levels in prediabetes patients.

OBJECTIVES:
1. To determine the serum levels of hsCRP in elderly prediabetes patients with early diabetic complications and to compare with diabetic patients without early diabetic complications and with normal subjects.
2. To determine whether these serum hsCRP level correlate with glycosylated haemoglobin levels and
albumin creatinine ratio in prediabetics with and without early diabetic changes.

**MATERIALS AND METHOD:** The study included Type 2 diabetic patients, as defined by the World Health Organisation (WHO). S6 diabetic patients attending the OPD of Endocrinology and Medicine departments of SIMS & RC from August to October 2014 were recruited. 24 normal subjects were selected a control group. All the subjects completed a standard questionnaire regarding current medication and duration of diabetes. The subjects in the age range 50-60 years and duration of diabetes more than 10 years were included for the study. Subjects with history of any significant infections, trauma, malignancy, smoking or on any anti-inflammatory drug or with BMI >30 were excluded from the study. Patients on dialysis were also excluded. Informed consent was taken from all the subjects. Ethical clearance was obtained from the institutional ethics committee.

After 12 hr of fasting, blood samples were collected from median cubital vein using commercially available vacutainers. The level of HbA1c was determined by borate affinity assay (NycoCard). The serum creatinine was estimated by modified Jaffe's method. The serum was stored at -20°C in Eppendorf tubes till samples were analysed for hsCRP.

The subjects submitted urine samples that had been collected at home over the previous 24h. Written instructions and careful explanation regarding the procedure for urine collection were given to each subject. Most of the patients with diabetes were familiar with the method for collecting urine at home. Nevertheless, a urine sample was discarded if there was any doubt with regard to its collection. The 24-h urine samples collected from each subject were used to determine the value of the urinary albumin excretion. Urine sample collected was analysed for albumin by turbidimetry method.

Patients were divided into 2 groups based on albumin creatinine ratio. Group 1 were diabetic patients with AC ratio between 30–300 mg/gm and group 2 were diabetic patients with AC ratio >300mg/gm. 24 age and sex matched subjects with no diabetes were recruited as controls.

**Measurement of hsCRP Concentration:** Blood samples were collected in tubes containing citric acid and stored at 20°C after centrifugation. hsCRP concentrations were measured using a latex-enhanced immunonephelometry (range0.05–10mg/l; BIOSYSTEMS)

**STATISTICAL ANALYSIS:** Data are given as Mean±SD. Means for basics characteristics and the laboratory data were computed for the case and control subjects and analysed using analysis of variance. hsCRP data were log transformed to stabilize the variance. Pearson correlation coefficient has been used to find the relationship between hsCRP and the following variables: HbA1c, AC ratio and serum creatinine.

**Statistical Software:** The Statistical software namely SPSS 11.0 and Systat 8.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate tables. The threshold of statistical significance was defined as p <0.01.

**RESULTS:** In our study there were no diabetic patients with our inclusion criteria with AC ratio<30mg/gm. Therefore, we grouped the diabetic patients into 2 groups. Group1 were diabetic patients with AC ratio between 30–300 mg/gm and group 2 were diabetic patients with AC ratio >300mg/gm. Table 1 shows the comparison of Basic characteristics and parameters in study groups. There is a significant increase (p<0.01) in serum hsCRP levels in diabetics with AC ratio >300mg/gm (mean=6.87±3.84) compared to diabetics with AC ratio 30-300mg/gm (mean=3.13±3.71) and normal controls (mean=2.35±2.64). HbA1c values showed a significant increase in diabetic with AC ratio>300mg/gm group compared to the other two groups. Table 3 shows the correlation of hsCRP with HbA1c, serum creatinine and urine albumin in diabetic patients and in healthy controls. The hsCRP levels show a positive moderate correlation with HbA1c and AC ratio diabetic patients. In healthy controls, hsCRP showed no significant correlation with any of the parameters.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>No diabetes</th>
<th>Total subjects with Pre Diabetes</th>
<th>Pre Diabetes with AC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>30-300 mg/gm</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Age (Years)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hsCRP (mg/l)</td>
<td>2.35±2.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HbA1c (%)</td>
<td>5.67±0.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urinary Albumin Creatinine ratio (mg/gm)</td>
<td>117.70±56.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. Creatinine (mg/dl)</td>
<td>0.87±0.12</td>
</tr>
</tbody>
</table>

**Table 1:** Comparison of Basic characteristics and parameters in study groups

Mean ± SD
significant changes cytokines production chronic diabetic presence <300mg/gm >300mg/gm.

**DISCUSSION:** In our study we compared serum hsCRP levels in pre diabetic and in healthy controls. Mean values of hsCRP showed a significant increase among pre-diabetic patients when compared with normal controls. There is an increase in hsCRP levels in diabetics with AC ratio >300mg/gm. compared to diabetics with AC ratio <300mg/gm and normal subjects. The results indicate the presence of inflammatory process in pre diabetics and pre diabetic with early diabetic complications. Therefore, inflammation reflects the severity of the disease and signifies the presence of ongoing disease process. These observations are consistent with previous studies.3,16

There are several possible mechanisms by which chronic low-degree inflammation might be induced in diabetes and its complications. In a hyperglycaemic condition, the concentration of advanced glycation end products increases. Advanced glycation end products have been shown to activate macrophages, increase oxidative stress, and upregulate the synthesis of interleukin-1, interleukin-6, and tumour necrosis factor, resulting in the production of CRP.17 Another possibility is that increases in CRP concentrations are related to adipose tissue-derived cytokines.18 However, the role of adipose tissue as a possible cause of the chronic inflammatory condition in patients with pre diabetic with and without early diabetic changes requires further investigation.

HbA1c showed significant difference among the three groups, indicating the role of poor glycaemic control in the development of early diabetic changes. We also found a significant positive correlation of serum hsCRP levels with HbA1c. Studies have shown similar association between hyperglycaemia and inflammation.19 It is known that glycation triggers the inflammatory process, leading to a rise in hsCRP levels. Thus, hsCRP can predict the onset of glycation induced inflammatory process secondary to poor glycaemic control.3

The present study also clearly shows that elevated hsCRP concentrations correlate with the AC ratio in diabetic patients. Therefore, low-grade inflammation may be a risk factor for the early diabetic complications in patients with pre diabetes. To evaluate this possibility, prospective studies are required.

**CONCLUSIONS:** From our study we conclude that serum hsCRP levels are increased in pre diabetic patients and correlates with HbA1c and albuminuria. Thus systemic inflammation has an important role in the pathogenesis of pre diabetic. There is a progressive increase in hsCRP levels in pre diabetic patients. Therefore, inflammation reflects the severity of the disease and signifies the presence of ongoing disease process. We also found a positive correlation between hsCRP and HbA1c, also between hsCRP and AC ratio among the diabetic groups. Thus, serum hsCRP and therefore inflammation is associated with glycaemic control and albuminuria. Thus, estimation of serum hsCRP levels and aiming at good glycaemic control helps in early intervention and prevention of further complications in pre diabetic with early diabetic changes patients. Interventions at early stages to reduce inflammation may preserve renal function in these individuals.

**REFERENCES:**


<table>
<thead>
<tr>
<th>Relationship Between</th>
<th>hsCRP</th>
<th>HbA1c</th>
<th>S. Creat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs. Microalbuminuria</td>
<td>t value</td>
<td>0.88</td>
<td>0.27*</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>0.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control vs. Macroalbuminuria</td>
<td>t value</td>
<td>0.94*</td>
<td>0.27*</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Micro vs. Macroalbuminuria</td>
<td>t value</td>
<td>0.91*</td>
<td>0.27*</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>0.007</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 2: Table Showing the Significance of Difference Between the Groups for The Parameters**

<table>
<thead>
<tr>
<th>Parameters in cases</th>
<th>HbA1c</th>
<th>S. Creat</th>
<th>AC Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP in cases</td>
<td>r value</td>
<td>0.38**</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>0.007</td>
<td>0.08</td>
</tr>
<tr>
<td>hsCRP in controls</td>
<td>r value</td>
<td>0.05</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>0.74</td>
<td>0.55</td>
</tr>
</tbody>
</table>

**Table 3: Correlation of hsCRP with HbA1c, Serum Creatinine and AC ratio in Pre Diabetic cases and controls**

*Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

r=Pearson’s correlation co-efficient.