

Acute Phase Reactant profile of subjects with Prediabetes and Type 2 Diabetes Mellitus.

Abstract:

Aim: The aim of the study is to examine the acute phase reactants in the backdrop of insulin resistance represented by prediabetic and overt diabetic states.

Study design: The study involves the evaluation of acute phase reactants at diverse glycemic stages.

Place and duration of the study: The study was undertaken at Rajarajeswari Medical college and Hospital, Bengaluru for around six months between July 2013 and December 2013.

Methodology: The study consists of three groups. We incorporated the World health organisation criteria for the diagnosis of prediabetes and type 2 diabetes mellitus. Accordingly forty seven adults were included in the first prediabetic group, the second group comprised of thirty seven type 2 diabetes mellitus patients. Thirty age and sex matched healthy volunteers constituted the third group. We intend to study High sensitive C reactive protein, serum albumin and serum uric acid levels, estimated by standard methods in the three groups.

Results: The Mean and standard deviation of HsCRP in prediabetic, T2DM and controls are 4.09 ± 1.29 , 3.38 ± 1.41 and 1.68 ± 0.51 mg//L respectively ($p=0.030$) in the present study. The serum albumin levels exhibit a statistically significant ($p=0.001$) downward trend from 4.67 ± 0.49 g/dl in the healthy controls to 4.01 ± 0.44 g/ dl in prediabetics and 3.96 ± 0.55 g/dl in diabetics. We have not noticed any statistically significant difference in the levels of uric acid among the three groups.

Conclusion: The acute phase reactant profile corresponds to the inflammatory environment and worsens as the disease progresses, in a metabolic disorder like T2DM, which is apparently evident in our study.

Key words: Acute phase reactants, Prediabetes, Type 2 Diabetes mellitus, Inflammation

Introduction

Inflammation is a response of vascularized tissues to infections and tissue damage that brings cells and molecules of host defence from the circulation to the sites where they are needed to eliminate the offending agents. The initial, rapid response is called the acute inflammation. When the initial response fails to clear the stimulus, the reaction progresses to a protracted type called the chronic inflammation¹. Some forms of chronic inflammation are involved in the pathogenesis of metabolic disorders like type 2 diabetes mellitus(T2DM), certain cancers and autoimmune

diseases². Recent studies have also substantiated the association of a subclinical low grade systemic inflammation with insulin resistance(IR) and atherogenesis.^{3,4}

Inflammation, acute or chronic is associated with cytokine-induced systemic reactions that are collectively called the acute phase reactions. Acute phase reactants(APR) have been defined as those whose plasma concentration increases(positive APR) or decreases (negative APR) by at-least 25 % during inflammatory disorders.⁵ The best known positive phase reactants are C-reactive protein, fibrinogen and serum amyloid A protein. Serum albumin and pre-albumin are the negative phase reactants⁶. Acute phase reactants have beneficial effects during acute inflammation, but prolonged production of these proteins in chronic inflammation exert deleterious effects

Insulin resistance, is one such consequence of prolonged low grade inflammation. Insulin resistance is predominant of all the sequel of events which terminate in development of type 2 diabetes mellitus. Prediabetes, which includes Impaired fasting glucose(IFG) and impaired glucose tolerance(IGT) is a stage between normal glucose tolerance and T2DM. It represents the advancing insulin resistance and failing beta cell function⁷. The interlink between inflammation and insulin resistance has not been clearly understood so far. However the association between inflammatory markers and insulin resistance has been accounted several times in the recent past^{8,9}.

Our aim in this study is to examine the pro-inflammatory markers in the backdrop of insulin resistance represented by prediabetic and overt diabetic states. The acute phase reactants we have chosen for our study includes High sensitive C-reactive protein(Hs-CRP), serum albumin and uric acid.

Materials and methods

This is a cross sectional study, we undertook at Rajarajeswari Medical college and Hospital, Bengaluru. Forty seven individuals identified as prediabetics according to World health organisation criteria¹⁰ constituted our first group. Thirty seven subjects, satisfying WHO diagnostic norms for the diagnosis of T2DM¹⁰ formed our second group. The control group consisted of thirty healthy volunteers of our Hospital. All the subjects of our study were between 20 and 65 years age group. Care was taken not to induct individuals with any acute or chronic illness and pregnant ladies. The approval from Institutional Ethics Committee and a written informed consent from participants of our research project was obtained.

After an overnight 12 hours fast, the venous blood sample was drawn from the subjects under all aseptic precautions. Fasting blood glucose (FBG), Hs-CRP, serum albumin and serum uric acid were estimated in this sample. After this 2 hour post-prandial venous blood sample was drawn and post prandial blood glucose(PPBG) was estimated.

69 Blood glucose was estimated by Glucose oxidase peroxidase method. HsCRP was estimated
70 by Latex enhanced immunoturbidimetric method. The elevated HsCRP was defined as levels above
71 3 mg%¹¹. Serum albumin was analysed by Bromocresol green methodology and serum uric acid by
72 uricase method . The estimations were done on fully automated analyzer, Mindray BS-300.

73

74 **Statistical Methods:** Descriptive and inferential statistical analysis has been carried out in the
75 present study. Results on continuous measurements are presented on Mean $\pm$ SD (Min-Max) and
76 results on categorical measurements are presented in Number (%). Significance is assessed at 5 %
77 level of significance. Analysis of variance (ANOVA) has been used to find the significance of study
78 parameters between three or more groups of patients. Chi-square/ Fisher Exact test has been used to
79 find the significance of study parameters on categorical scale between two or more groups.

80 **Results:**

81 The individuals in our study were assigned the group to which they belong based the two
82 important biochemical parameters, the fasting blood glucose and the post prandial blood glucose.
83 Therefore the mean $\pm$ standard deviation of FBG and PPBG values significantly varied in the three
84 groups. The principle focus here is to study the acute phase reactant profile of the individuals in the
85 three groups. The mean $\pm$ standard deviation values of HsCRP, serum albumin and serum uric acid
86 are tabulated and compared to assess the statistical significance difference based on the p value.
87 Table 1 is thus a compilation of the mean $\pm$ standard deviation and p values of the study parameters
88 in the three groups.

89

90 **Table 1:** Comparison of study variables in three groups studied

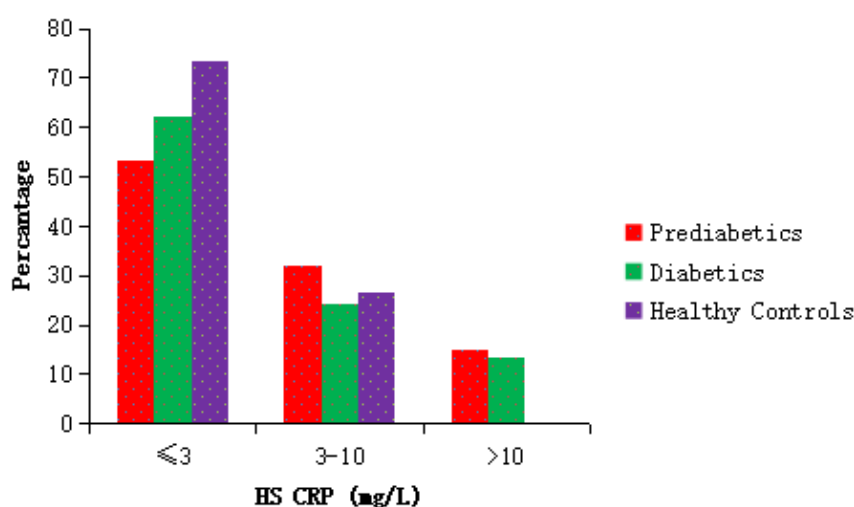
	Prediabetics (n=47)	Diabetics (n=37)	Healthy Controls (n=30)	P value
FBG (mg/dl)	106.30 $\pm$ 13.05	165.8 $\pm$ 774.18	89.03 $\pm$ 11.83	0.006**
PPBG (mg/dl)	165.43 $\pm$ 18.03	252.30 $\pm$ 88.63	106.77 $\pm$ 12.78	<0.001**
Hs CRP(mg/L)	4.09 $\pm$ 1.29	3.38 $\pm$ 1.41	1.68 $\pm$ 0.51	0.030*
UA (mg/dl)	3.98 $\pm$ 1.80	4.17 $\pm$ 1.39	4.33 $\pm$ 1.49	0.638
Albumin (g/dl)	4.01 $\pm$ 0.44	3.96 $\pm$ 0.55	4.67 $\pm$ 0.49	<0.001**

91 **Correlation is significant at the 0.01 level

*Correlation is significant at the 0.05 level

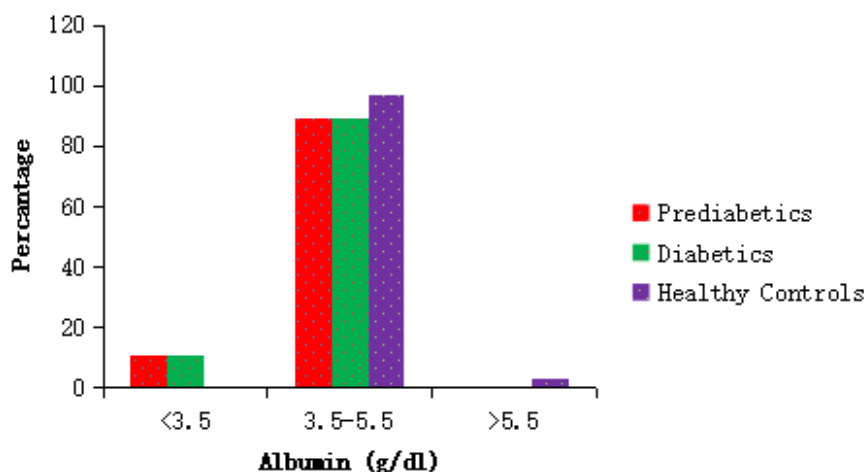
High sensitive C-reactive protein is an acute phase reactant, however it is also an established cardiovascular risk marker. The reference value of less than 1, 1 to 3 and greater than 3 mg/L, correspond to approximate tertiles of the CRP distribution of individuals into low, moderate and high cardiovascular risk groups¹². Figure 1 is a graphical representation of HsCRP levels classified into <1, 1-3 and >3 mg/L in the three groups .

Figure 1: Graph representing low, moderate and high HsCRP levels within the three study groups



The reference range of serum albumin in adults is 3.5 to 5.5 g/dl. Albumin is a negative phase reactant and the levels are expected to fall in a inflammatory background .The subjects in each group were divided further based on albumin levels as follows. Serum albumin less than 3.5, 3.5 to 5.5 and greater than 5.5 g/dl. Figure 2 is the representation of the same.

Figure 2: Graphical depiction of low, normal and elevated serum albumin levels within the three study groups



126 **Discussion:**

127 India harbors two sections of population representing two different ends of a spectrum. One,
128 the rural India which is still battling poverty and malnutrition and the other, over-nourished urban
129 Indians who are falling prey to obesity and its complications. Chronic over nutrition might be a
130 pro-inflammatory state with oxidative stress¹³. Over a period of time this may result in insulin
131 resistance and lead to prediabetic state, which when goes unnoticed, culminates into frank type 2
132 diabetes mellitus and its related complications.

133 The evaluation of acute phase reactants at the different stages of glycemia may give us a
134 clue about the course of inflammation and that is the primary intention of our study.

135 For decades now, C reactive protein, is a well established inflammatory marker. The specific
136 ligand for CRP is the pneumo-coccal 'C' polysaccharide as phosphocholine, part of the teichoic acid
137 of the pneumococcal cell wall, hence the name C-reactive protein. It is synthesized in the liver.
138 CRP consists of five identical, noncovalently associated 23- kDa protomers arranged symmetrically
139 around a central pore.¹⁴ Minor elevation in CRP levels in the blood are detected by sensitive
140 biochemical assay and are designated as high sensitive C-reactive protein. Hs-CRP is a
141 cardiovascular risk marker.

142 The Mean and SD of Hs CRP in prediabetic, T2DM and controls are 4.09 ± 1.29 , 3.38 ± 1.41
143 and 1.68 ± 0.51 mg/L respectively in the present study. A statistically significant ($p=0.030$) and
144 orderly increase is observed in the levels from controls, to prediabetic to diabetic group. Our
145 findings are consistent with the observations made by Gupta AK et al and Sabanayagam C et al who
146 have shown a marked increase in HsCRP levels in prediabetics when compared to
147 normoglycemics^{15,16}. Freeman DJ et al and Pradhan AD in their studies have noticed a marked
148 increase in CRP levels in diabetics than in healthy individuals^{17, 18}. These findings authenticate the
149 presence of an underlying inflammatory milieu during the evolution of T2DM. From Figure 1, it is
150 further evident that there are individuals with HsCRP levels of >10 mg/L in pre-diabetic and
151 diabetic (15 % & 14 % respectively) but not in healthy control group. The data portrays that people
152 with diabetes and pre-diabetes are more prone to cardio-vascular disease risk than euglycemics.

153 Albumin, a small globular protein with a molecular weight of 66.3kDa is the most abundant
154 protein present in the plasma. It is a negative phase protein. Hypoalbuminemia is a common feature
155 of acute or chronic inflammation. The albumin levels exhibit a statistically significant ($p=0.001$)
156 downward trend from 4.67 ± 0.49 g/dl in the healthy controls to 4.01 ± 0.44 g/dl in prediabetics and
157 3.96 ± 0.55 g/dl in diabetics. The albumin levels decrease during inflammation due to one of the
158 following reasons: Hemodilution, loss into extra vascular space, increased consumption by cells and
159 decreased synthesis¹⁹. Figure 2 illustrates hypoalbuminemia (<3.5 g/dl) of 11% in both prediabetic
160 and diabetic groups.

Uric acid, an end product of purine catabolism, possesses anti-oxidant properties. Researchers have opined that the elevated uric acid levels in cardiovascular diseases might be a compensatory response designed to counteract excessive oxidative stress. Studies have also proven a significant association between high uric acid levels and pro inflammatory mechanisms^{20, 21}. In our study we have not noticed any statistically significant difference in the levels of uric acid among the three groups.

To conclude, the pro-inflammatory markers, otherwise called the acute phase reactant profile corresponds to the inflammatory environment and worsens as the disease progresses in a metabolic disorder like T2DM, as is apparently evident in our study.

Limitation of our study: As the sample size of our study was small we could not look for a correlation between the acute phase reactants and dysglycemia. We recommend similar venture in a larger population.

Competing interests: There was no competing interests between the authors of this study.

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