International Journal of Biochemistry Research & Review

19(4): 1-6, 2017; Article no.IJBCRR.37785
ISSN: 2231-086X, NLM ID: 101654445

Acute Phase Reactant Profile of Subjects with Prediabetes and Type 2 Diabetes Mellitus

N. Chandrika[1*] and S. M. R. Usha[2]

[1]Department of Biochemistry, Chamarajanagar Institute of Medical Sciences, Chamarajanagar, India.
[2]Department of Biochemistry, Rajarajeswari Medical College and Hospital, Bengaluru, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author NC designed the study, wrote the protocol and the first draft of the manuscript. Author SMRU performed the statistical analysis, managed the literature searches and the analyses of the study. Both the authors have read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2017/37785
Editor(s):
(1) Mohamed Fawzy Ramadan Hassanien, Biochemistry Department, Faculty of Agriculture, Zagazig University, Egypt.
Reviewers:
(1) Yuji Aoki, Matsumoto Medical Center Matsumoto Hospital, Japan.
(2) Keagile Bati, University of Botswana, Botswana.
(3) Takashi Ikeno, National Center of Neurology and Psychiatry, Japan.
Complete Peer review History: http://www.sciencedomain.org/review-history/21727

Original Research Article

Received 28th October 2017
Accepted 2nd November 2017
Published 3rd November 2017

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: Cross sectional study
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 constitued 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

**Corresponding author: E-mail: dr.chandrikar@gmail.com;*

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.

Keywords: Acute phase reactants; prediabetes; type 2 diabetes mellitus; inflammation.

1. 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 inflammation [1]. Some forms of chronic inflammation are involved in the pathogenesis of metabolic disorders like type 2 diabetes mellitus (T2DM), certain cancers and autoimmune diseases [2]. 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 reactants [6]. 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 function [7]. 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.

2. 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 criteria [10] constituted our first group. Thirty seven subjects, satisfying WHO diagnostic norms for the diagnosis of T2DM [10] 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 the fasting sample was drawn, the subjects of all the three study groups had staple South Indian breakfast which usually consists of two idlis with coconut chutney. The total calorie content of the food they had was approximately around 200 Calories. Exactly two hours after breakfast a post-prandial venous blood sample was drawn and post prandial blood glucose (PPBG) was estimated.

Blood glucose was estimated by Glucose oxidase peroxidase method. HsCRP was estimated by Latex enhanced immunoturbidi-

metric method. The elevated HsCRP was defined as levels above 3 mg/L [11]. Serum albumin was analysed by Bromocresol green methodology and serum uric acid by uricase method. The estimations were done on fully automated analyzer, Mindray BS-300.

2.1 Statistical Methods

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5% level of significance. Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of patients.

3. RESULTS

The mean ± standard deviation of the age of the people in different groups of our study is as follows. The mean ± SD of age in prediabetic group is 49.8 ± 14.4, diabetic group is 50.32 ± 10.07 and that of control group is 45.6 ± 12.95 years. The diabetic and healthy control group is constituted by 60% females and 40% males each. In prediabetic group 49% of the study subjects are females and 51% are males**.** The individuals in our study were assigned the group to which they belong based the two important biochemical parameters, the fasting blood glucose and the post prandial blood glucose. Therefore the mean ± standard deviation of FBG and PPBG values significantly varied in the three groups. The principle focus here is to study the acute phase reactant profile of the individuals in the three groups. The mean ± standard deviation values of HsCRP, serum albumin and serum uric acid are tabulated and compared to assess the statistical significance difference based on the p value. Table 1 is thus a compilation of the mean ± standard deviation and p values of the study parameters in the three groups.

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 groups [12]. Fig. 1 is a graphical representation of HsCRP levels classified into ≤ 3, 3-10 and >10 mg/L, the distribution which we have used in the three groups of our study.

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±13.05	165.8± 774.18	89.03±11.83	0.006**
PPBG (mg/dl)	165.43±18.03	252.30±88.63	106.77±12.78	<0.001**
Hs CRP(mg/L)	4.09±1.29	3.38±1.41	1.68±0.51	0.030*
UA (mg/dl)	3.98±1.80	4.17±1.39	4.33±1.49	0.638
Albumin (g/dl)	4.01±0.44	3.96±0.55	4.67±0.49	<0.001**

** P value =0.05 suggest significance; **P < 0.01 level suggests strong significance*

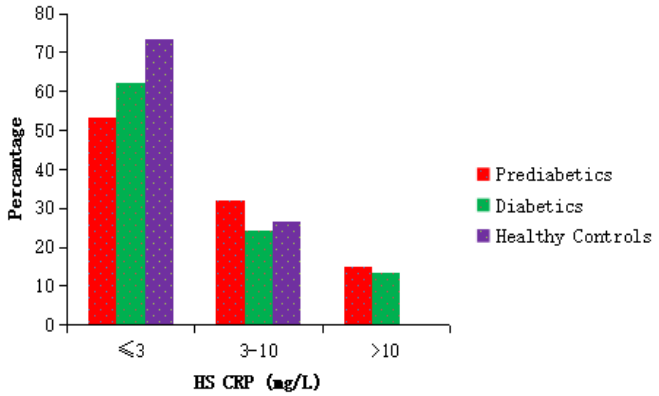

Fig. 1. Graph representing low, moderate and high HsCRP levels within the three study groups

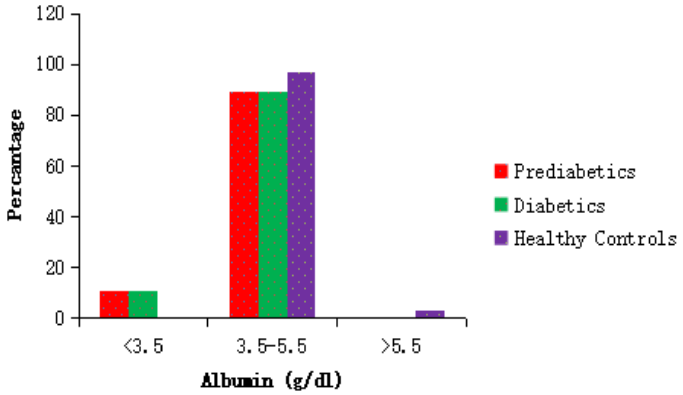

Fig. 2. Graphical depiction of low, normal and elevated serum albumin levels within the three study groups

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. Fig. 2 above is the representation of the same.

4. DISCUSSION

India harbors two sections of population representing two different ends of a spectrum. One, the rural India which is still battling poverty and malnutrition and the other, over-nornished urban Indians who are falling prey to obesity and its complications. Chronic over nutrition might be a pro-inflammatory state with oxidative stress [13]. Over a period of time this may result in insulin resistance and lead to prediabetic state, which when goes unnoticed, culminates into frank type 2 diabetes mellitus and its related complications.

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

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

The Mean and SD of Hs CRP in prediabetic, T2DM and controls are 4.09±1.29, 3.38±1.41 and 1.68±0.51 mg//L respectively in the present study. A statistically significant (p=0.030) and orderly increase is observed in the levels from controls, to prediabetic to diabetic group. Our findings are consistent with the observations made by Gupta AK et al and Sabanayagam C et al who have shown a marked increase in HsCRP levels in prediabetics when compared to normoglycemics [15,16]. Freeman DJ et al. and Pradhan AD in their studies have noticed a marked increase in CRP levels in diabetics than in healthy individuals [17,18]. These finding authenticates the presence of an underlying inflammatory milieu during the evolution of T2DM. Fig. 1 gives a further insight into HsCRP variations within the study groups. Levels of HsCRP of ≤ 3 mg/ L denotes low risk of cardiovascular disease (CVD) risk. In our study, 25 (53%) people in prediabetic group, 23(62%) in diabetic and 22(73%) individuals of healthy control group fell under this category. There are 15 (32%) individuals in prediabetic, 9 (24%) in diabetic and 8 (27%) in control group who had Hs CRP levels between 3 an d 10 mg/L. HsCRP levels of > 10 mg L signifies high risk for CVD. From Fig. 1, it is further evident that 7 (15%) subjects of prediabetes and 5 (14%) in diabetic group are at high risk of developing CVD but none among the healthy controls are at high risk for CVD. This proves that people with diabetes and pre-diabetes are more prone to cardio-vascular disease than euglycemics.

Albumin, a small globular protein with a molecular weight of 66.3kDa is the most abundant protein present in the plasma. It is a negative phase protein. Hypoalbuminemia is a common feature of acute or chronic inflammation. The 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. The albumin levels decrease during inflammation due one of the following reasons: Hemodilution, loss into extra vascular space, increased consumption by cells and decreased synthesis [19]. An interesting observation of our study is that the distribution of serum albumin levels follows an identical pattern in prediabetic and diabetic groups. This is demonstrated in Fig. 2. Hypoalbuminemia (<3.5 g/dl) which depicts an underlying inflammation is seen as many as 5(11%) and 4 (11%) subjects in prediabetic and diabetic group respectively. Similarly albumin levels between 3.5 and 5.5 g/dl was present in 89% of individuals in both prediabetic and diabetic groups. There is only one person in the control group with albumin level > 5.5 g/dl, but none in the remaining two 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]. The findings of our study are every much in lines with the observation made by Jing Cheng et al. [22] in their project involving a large study group. Jing Cheng et al have reported that serum uric acid levels was significantly higher in higher insulin resistance, similarly in our study, though we have not noticed any statistical significance, the mean ± SD of uric acid is more in diabetics(4.17±1.39 mg/dl) than in prediabetics (3.98±1.80 mg/dl). However in the control the uric acid levels is 4.33±1.49 mg/dl which is more than that compared to the other two groups. This finding has left us perplexed and this aspect of the study needs further investigation.

5. CONCLUSION

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.

6. 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

Authors have declared that no competing interests exist.

REFERENCES

1. Inflammation and repair. In: Kumar V, Abbas AK, Aster JC. (eds.) Robbins Basic pathology. 10th Edition. Philadelphia. Elsevier. 2018;57-87.
2. Khansari N, Shakiba Y, Mahdi MM. Chronic inflammation and oxidative stress as a major cause of age- related diseases and cancer. Inflammation & Allergy Drug Discovery. 2009;3(1):73-80.
3. Steven M. Haffner. Pre-diabetes, insulin resistance, inflammation and CVD risk. Diabetes Research and Clinical Practice. 2003;61:S9- S18.
4. Gonza´lez AS, Guerrero DB, Bun˜o Soto M, Pe´rtega D´az S, Martinez-Olmos M, Vidal O. Metabolic syndrome, insulin resistance and the inflammation markers C-reactive protein and ferritin. European Journal of Clinical Nutrition. 2006;60:802–809.
5. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999;340: 448-454.
6. Rifai N, Warnick GR, Remaley AT. Lipids, lipoproteins, Apoproteins, and other cardiovascular risk factors. In: Burtis CA, Ashwood ER, Bruns DE. (eds.) Teitz Fundamentals of Clinical Chemistry. 6th Edition. Elsevier. 2008;427-429.
7. International Diabetes Federation, Diabetes Atlas, 7th Edition; 2015.
8 Festa A, Hanley AJ, Tracy RP, D'Agostino R, Haffner SM. Inflammation in the prediabetic state is related to increased insulin resistance rather than decreased insulinsecretion. Circulation. 2003;108: 1822–1830.

9. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. Diabetes. 2002;51:455–461.
10. World Health Organisation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia.
Available:http://www.who.int/diabetes/publications/Definition and diagnosis of diabetes
(Accessed on 05.10.2017)
11. Pu LJ, Lu L, Xu XW, Zhang RY, Zhang Q, Zhang JS, et al. Value of serum glycated albumin and high-sensitivity C-reactive protein levels in the prediction of presence of coronary artery disease in patients with type 2 diabetes. Cardiovasc Diabetol. 2006;5:27.
12. Rifai N, Ridker PM. Population distribution of C-reactive protein in apparently healthy men and women in the United States: Implication for clinical interpretation. Clin Chem. 2003;49:666-669.
13. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: The link between insulin resistance, obesity and diabetes. Trends Immunol. 2004;25(1):4-7.
14. Black S, Kushner I. C-reactive protein. The Journal of Biological Chemistry. 2004; 279(47):48487–48490.
15. Gupta AK, Johnson WD. Prediabetes and prehypertension in disease free obese adults correlate with an exacerbated systemic proinflammatory milieu. Journal of Inflammation. 2010;7:36.
16. Sabanayagan C, Shankar A, Lim SC, Lee J, Tai ES, Wong TY. Serum C-reactive protein level and prediabetes in two Asian populations. Diabetologia; 2011.
DOI: 10.1007/s00125-011-2052-5
17. Freeman DJ, Norrie J, Caslake MJ, Gaw A, Ford I, Lowe GDO, O'Reilly DSJ, Packard, CJ and Sattar N. C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study for the West of Scotland Coronary Prevention Study Group. Diabetes. 2002;51:1596-1600.
18. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, Interleukin-6 and risk of developing type 2 diabetes mellitus. Diabetes. 2002;51:327-334.
19. Johnson AM. Amino acids and proteins. In: Burtis CA, Ashwood ER, Bruns DE, (eds.). Teitz Fundamentals of Clinical Chemistry. 6th Edition. Elsevier. 2008;297-298.
20. Carnethon MR, Fortmann SP, Palaniappan L, Duncan BB, Schmidt MI, Chambless LE. Risk factors for progression to incident hyperinsulinemia: The atherosclerosis risk in communities study, 1987–1998. Am J Epidemiol. 2003;158:1058–1067.
21. Nakanishi N, Okamoto M, Yoshida H, Matsuo Y, Suzuki K, Tatara K. Serum uric acid and risk for development of hypertension and impaired fasting glucose or Type II diabetes in Japanese male office workers. Eur J Epidemiol. 2003;18:523–530.
22. Cheng J, Reynolds K, Wildman RP, Whelton PK, Hamm LL, He J, Muntner P. Association Between Inflammation and Insulin Resistance in U.S. Nondiabetic Adults. Diabetes Care. 2004;27:2960–2968.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21727