Original Article

Biochemistry

Acute Phase Reactants in Type 2 Diabetes Mellitus and Their Correlation with the Duration of Diabetes Mellitus

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ABSTRACT

Introduction: Poorly controlled blood glucose levels can lead to complications in type 2 diabetes mellitus. The risk of the complications increases with an increase in the duration of hyperglycaemia. Numerous studies have shown the association between chronic subclinical inflammation and the risk of developing type 2 diabetes. Acute phase reactants are the markers of inflammation. In our study, we assessed the correlation of the levels of the acute phase reactants with an increase in the duration of diabetes mellitus. We also studied the correlation between these acute phase reactants with the traditional risk predictors of diabetic complications.

Materials and Methods: This was a case control study which included 60 uncontrolled, type 2, diabetes mellitus cases and 30 non-diabetic, apparently normal controls. The cases were classified into two groups. Group I comprised of 30 cases which had a duration of diabetes of less than 10 years and group II comprised of 30 cases which had a duration of diabetes of more than 10 years. Three acute phase reactants; C reactive protein, ceruloplasmin and total sialic acid were assessed as the markers of inflammation. Cardiovascular risk was assessed by measuring the lipid profile. 24 hour urinary albumin was measured as a marker of incipient diabetic nephropathy.

Results: A significant increase was seen in all the three acute phase reactants in group I as compared to those in the controls (P< 0.01). The increase in these acute phase reactants was more significant (P< 0.001) in group II as compared to that in group I. There was a significant positive correlation between all the acute phase reactants , C reactive protein (r=0.9, P<0.001), ceruloplasmin (r=0.3, P<0.001) and total sialic acid (r=0.9, P<0.001) and the LDL:HDL cholesterol ratio in group II. A significant positive correlation was also seen between C reactive protein (r=0.5, P<0.001), ceruloplasmin (r=0.9, P<0.001) and total sialic acid (r=0.9, P<0.001) and the 24 hour urinary albumin in group II.

Conclusion: There was a significant increase in the level of the acute phase reactants with an increase in the duration of diabetes mellitus. There was a significant correlation between inflammation and the diabetic complications.

Key Words: Acute phase reactants, Type 2 diabetes mellitus, Inflammation, Diabetic complications

INTRODUCTION

Diabetes mellitus (DM) is a heterogenous, metabolic disease which is characterized by hyperglycaemia and long term complications. The late complications of diabetes are: (a) microangiopathy i.e. abnormalities of the small arteries which include diabetic nephropathy, retinopathy, neuropathy and (b) macroangiopathy i.e. abnormalities of the large arteries which include coronary heart disease and peripheral vascular disease. The risk of the chronic complications increases as a function of the duration of hyperglycaemia. The complications usually become apparent in the second decade of the hyperglycaemia [1].

Acute phase reactants (APRs) are the markers of inflammation. They are synthesized in response to tissue damage and inflammation. During inflammation, their concentrations increase thousand fold over the normal levels. They are mainly produced by hepatocytes, but they can also be synthesized by adipocytes, fibroblasts, endothelial cells, etc [2].

Recent studies have shown that low grade inflammation is associated with the risk of developing type 2 DM [3].There are only few reports regarding the role of inflammation in diabetic complications. Considering this fact, the present study was carried out to find out the changes in the levels of the acute phase reactants with an increase in the duration of diabetes mellitus and their role in the diabetic complications.

MATERIALS AND METHODS

This study was carried out in the Department of Biochemistry, Indira Gandhi Government Medical College, Nagpur. This case control study was carried out on a total of 90 subjects. 30 healthy and apparently normal, non-diabetic subjects were selected as the controls. 60 cases of uncontrolled type 2 diabetes mellitus were selected, which were diagnosed on the basis of the glycosylated haemoglobin levels (HbA1c >6.5%) [4]. The diabetic cases were recruited from the Diabetic OPD, Department of Medicine, Indira Gandhi Government Medical College, Nagpur. Out of these 60 cases of DM, 30 cases had DM for less than 10 years, which were categorized as group I. The rest of the 30 cases had DM for more than 10 years, which were categorized as group II. The subjects were of both sexes, who were in the age group of 30-60 years. Subjects with any inflammatory disease, those who were on statin therapy, smokers, women who were taking medications which contained estrogen and pregnant women were excluded from the study.

A requisite clearance from the institutional ethical committee was obtained. Fasting blood samples were collected from all the parti-

cipants after taking their written informed consent. Haemolyzed and lipaemic samples were excluded. The blood samples were analyzed for glycosylated haemoglobin by the cation-exchange resin method. In this method, a haemolysed preparation of whole blood was mixed continuously for 5 minutes with a weakly binding cation exchange resin. During this mixing, the non-glycosylated haemoglobin binds to the ion exchange resin, leaving the gycosylated haemoglobin(GHb) free in the supernatant. The percent glycosylated haemoglobin was determined by measuring the absorbances of the GHb fraction and the total haemoglobin fraction. The ratio of the absorbances of the GHb fraction and the total haemoglobin fraction of the control and test was used to calculate the percent glycosylated haemoglobin of the sample. The serum C-reactive protein (CRP) was analyzed by the turbilatex method (kit- MERCK laboratory).In this method, the latex particles which were coated with specific human anti-CRP were agglutinated when they were mixed with samples which contained CRP. This agglutination caused an absorbance change which could be quantified by comparision from a calibrator of known CRP concentration. The determination of serum ceruloplasmin (kit- MERCK laboratory) is based on the reaction between ceruloplasmin as an antigen and the specific antiserum as the antibody. This reaction forms an insoluble complex which produces a turbidity which is measured spectrophotometrically. The serum total sialic acid was measured by a chemical method which was used by Plucinsky MC et al [5], serum total cholesterol and triglycerides were evaluated by an enzymatic method, serum HDL cholesterol was evaluated by phosphotungstate precipitation followed by an enzymatic method and serum LDL and VLDL cholesterol were evaluated by using Friedewald [6] formula. Also, 24 hour urine samples were collected and analyzed for urinary albumin by the pyrogallol red method. All the parameters were analyzed by using a semiautomatic analyzer (Transasia Erba Chem–5 Plus).

STATISTICAL ANALYSIS

Statistical analysis was done by using the Graph Pad Prism software. The data was expressed as mean ± SD. The significance of the differences in the values of the parameters among the controls and group I and group II was evaluated by using ANOVA (Analysis of Variance). The comparisons between the two groups were done by applying the Student's 't' test. Pearson's correlation coefficient was employed to find out the correlations between the three acute phase reactants and the LDL:HDL ratio and the 24 hour urinary albumin in group II.

RESULTS

We observed the significance of the differences (P<0.001) in the values of all the parameters in the controls and group I and group II by using ANOVA. All the three acute phase reactants; C reactive protein, ceruloplasmin and total sialic acid were significantly increased in group I as compared to the controls (P< 0.01). The increase in these acute phase reactants was more significant (P< 0.001) in group II as compared to group I . 24 hour urinary albumin was significantly increased (P<0.01) in group I as compared to the controls. The increase in urinary albumin was more significant (P<0.001) in group II as compared to that in group I. The serum total cholesterol, LDL cholesterol (LDL-C) and VLDL cholesterol levels showed a significant increase (p<0.01) in group I as compared to those in the controls and a highly significant increase (P<0.001) in group II as compared to those in group I. The serum HDL cholesterol (HDL-C) level was significantly lower in group I (P<0.01) as compared to that in the controls. The decease in the HDL-C level was more significant (P<0.001) in group II as compared to that in group I. Also in our study, the LDL-C : HDL-C ratio was significantly increased (P<0.01) in group I as compared to that in the controls and it was much more significantly (P<0.001) increased in group II as compared to group I. There was a significant positive correlation between all acute phase reactants ; C reactive protein (r=0.9, P<0.001), ceruloplasmin (r=0.3, P<0.001) and total sialic acid (r=0.9, P<0.001) and the LDL:HDL cholesterol ratio in group II. A significant positive correlation was also seen between C reactive protein (r=0.5, P<0.001) , ceruloplasmin (r=0.9, P<0.001) and total sialic acid (r=0.9, P<0.001) and the 24 hour urinary albumin in group II.

DISCUSSION

In this study, all the three acute phase reactants showed a significant increase in the group I diabetes cases as compared to the controls [Table/Fig-1], which was in accordance with the reports of previous studies by Caparevic Z et al [7], Diamond M et al [8] and Gavella M et al [9]. In the last few years, numerous studies have shown that low grade inflammation is associated with the risk of developing type 2 diabetes. Furthermore, nowadays, it has been accepted that chronic subclinical inflammation is a part of the

Biochemical parameters	Normal values	Controls (n=30)	Group I (n=30)	Group II (n=30)
Glycosylated hemoglobin, HbA1c (%)	< 6.5	5 .4±0.3	8.8±0.7	9.1±0.8
Serum C –reactive protein (mg/l)	< 1	0.72±0.37	0.95±0.19*	6.86±2.89**
Serum ceruloplasmin (mg/dl)	25- 43	44±7	49±5*	78±4**
Serum total Sialic Acid (mg/dl)	40-60	56±6	60±3*	80±5**
Urinary albumin (mg/day)	< 30	5±4	19±6*	274±78**
Serum total cholesterol (mg/dl)	< 200	152±23	171±22*	264±28**
Serum triglyceride (mg/dl)	< 160	142±15	152±9*	250±32**
Serum HDL cholesterol (mg/ dl)	> 40	47±3	43±4#	37±3##
Serum VLDL Cholesterol (mg/dl)	< 32	28±3	30±1*	49±6**
Serum LDL cholesterol (mg/dl)	< 100	74±22	78±8*	177±26**
LDL-C :HDL-C ratio	< 2	1.58	1.79*	4.76**

[Table/Fig-1]: Biochemical profile of controls, group I and group II
* P < 0.01 Significant increase
** P < 0.001 Highly significant increase
# P < 0.01 Significant decrease
P < 0.001 Highly significant decrease

insulin resistance syndrome. The mechanisms by which chronic inflammation can evoke type 2 diabetes are not clear. However, it is known that adipose tissue can synthesize and release the main pro-inflammatory cytokines, tumour necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6), and that the inflammatory markers are associated with the body fat mass. The pro-inflammatory cytokines and acute phase reactants are involved in multiple metabolic pathways which are relevant to insulin resistance, including insulin regulation, reactive oxygen species, lipoprotein lipase action and adipocyte function.Therefore, the activated innate immunity and inflammation are relevant factors in the pathogenesis of diabetes, with convincing data that type 2 diabetes includes an inflammatory component [3].

We observed that there was a rise in the level of all the three acute phase reactants with an increase in the duration of diabetes mellitus [Table/Fig-1]. As DM is a chronic inflammatory state, an aberrant continuation of some aspects of the acute phase response may lead to the underlying tissue damage that accompanies the disease and can contribute to further complications [2].

According to Tan KC et al [10], chronic hyperglycaemia in uncontrolled type 2 diabetic patients causes the non enzymatic glycation of proteins, which leads to the formation of advanced glycation end products (AGEs). These AGEs can trigger the inflammatory response and are implicated in the pathogenesis of many complications of diabetes mellitus.

According to Wright E et al [11], oxidative stress through the production of reactive oxygen species (ROS) has been proposed as the root cause which underlies the development of insulin resistance, β cell dysfunction, impaired glucose tolerance and type 2 DM and its complications. The markers of inflammation, the well recognized manifestations of oxidative stress, have also been observed to be increased in response to the intermittent, elevated glucose levels. One of the many sequelae which lead to the generation of ROS is the cytokine induced stimulation of the acute phase reactant synthesis by the liver.

The risk of the chronic complications in diabetes mellitus increases as a function of the duration of hyperglycaemia; they usually become apparent in the second decade of the hyperglycaemia. Glomerular hyperfusion and renal hypertrophy occur in the first years after the onset of diabetes mellitus and cause an increase in the glomerular filtration rate. During the first five years of diabetes mellitus, thickening of the glomerular basement membrane, glomerular hypertrophy and mesangial volume expansion occur, as the glomerular filtration rate returns to normal. After 5 to 10 years, about 40% of the individuals begin to excrete small amounts of albumin in the urine. Microalbuminuria is defined as a urinary albumin excretion of 30-300 mg/day. The Group II cases in our study had microalbuminuria (urinary albumin 274 +78 mg/day). The appearance of microalbminuria is a very important predictor of incipient diabetic nephroathy [1].

The Group II cases in our study showed dyslipidaemia. According to Framingham's study [12], the persons with an LDL-C : HDL-C ratio which was greater than 5 were at a high risk of developing coronary heart disease (CHD) and those with a ratio between 2 - 5 were at an intermediate risk of developing CHD. So, in comparison with Framingham's study, the group II diabetics (LDL-C: HDL-C ratio= 4.76) were at an intermediate to high risk of developing CHD. This showed that the group II diabetics with a disease duration of more than 10 years were prone to renal and cardiovascular complications.

We observed a significant positive correlation between all the three acute phase reactants and the LDL-C:HDL-C ratio and also the 24 hour urinary albumin in group II diabetics. Thus, our study revealed that there was a significant correlation between inflammation and the diabetic complications. Nayak BS et al [13], Liao L et al [14], Panichi V et al [15], Pu L J et al [16] and Chen J et al [17] also demonstrated that the diabetic complications exhibited a sign of inflammation.

In conclusion, the pathogenetic vision of diabetes mellitus has changed in the last few years, with inflammatory pathways playing pivotal roles in the development and progression of the diabetic complications. These new pathogenic factors can lead to a consideration of new therapeutic approaches. The modulation of inflammatory processes in the setting of diabetes is nowadays a matter of great interest. It is possible that in the coming years, the hope of new therapeutic strategies which are based on anti-inflammatory properties, with beneficial actions on the diabetic complications, can be translated into real clinical treatments.

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