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Acute phase proteins in newly diagnosed diabetics.

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Abstract

A cytokine-mediated acute phase response is observed to be closely involved in the pathogenesis of type 2 diabetes. The role of inflammation in type 1 diabetes is contradictory. Since Indians are at high risk of developing diabetes, we tested this hypothesis by estimating circulating acute phase proteins in both type 1 (T-1) and type 2 (T-2) diabetic patients.

The acute phase proteins, α1- antitrypsin, α1- acid glycoprotein, ceruloplasmin and fibrinogen were estimated in the plasma in newly diagnosed 12 T-1 and 25 T-2 cases. Thirty normal controls to match the age and sex of the test groups were also studied. The levels of these proteins were correlated with their BMI and random plasma glucose values.

In comparison with the controls, T-1 cases showed significantly higher levels of the acute phase proteins (except α1- acid glycoprotein). The values of all the four proteins studied were significantly elevated in the T-2 patients ($p<.00001$). Except for ceruloplasmin levels, T-2 cases had significantly higher values when compared to the T-1 cases ($p<.00001$). Interestingly, no correlation was found with BMI or the degree of hyperglycemia in either of the types.

A low grade inflammatory process is definitely implicated in the pathogenesis of both type 1 and type 2 diabetes. This line of pathological basis should be further explored for diagnosis, management and follow up.

Introduction

Diabetes mellitus has emerged as a major health challenge of the 21st century. The disease has assumed epidemic proportions globally. WHO projects that, by the year 2025 about 300 million people will have diabetes most of who will inhabit China, India and the United States [1]. For centuries we have known the existence of two types of diabetes; the type 1, where the basic defect is an absolute deficiency of insulin due to an autoimmune destruction of the β cells and the type 2 diabetes where there is decreased secretion of insulin or an increased resistance to the action of insulin by the insulin sensitive tissues. This simple classification was complicated by the emergence of a spectrum of overlapping patient characteristics and the disease proper. Thus sub classifications like MODY (Maturity onset diabetes of the young), LADA (Latent autoimmune diabetes in adults) and several others came into existence. In search of better understanding of the pathogenesis, one that paved way for further research is the role of activated innate immunity in the development of type 2 and possibly type 1 diabetes [2,3]. Studies have shown that circulating inflammatory markers like C-reactive protein (CRP) and fibrinogen and their mediators interleukin 1 (IL-1), IL-6, and Tumour necrosis factor - α (TNFα) are higher in type 2 diabetics [4,5]. Similar studies in type 1 diabetics are contradictory [3,6,7]. Since Indians are at a high risk of developing diabetes both genetically and environmentally [8,9], we estimated a few of the lesser studied acute phase proteins, α-1 antitrypsin, α-1 acid glycoprotein, ceruloplasmin and fibrinogen in type 1 and type 2 newly diagnosed diabetic patients.

Materials and Methods

Patients visiting the Kasturba Medical College Hospitals with features of previously undiagnosed diabetes were examined by the physicians. Of the confirmed cases, patients with a history of chronic inflammatory diseases, episodes of recent acute inflammation, smokers, alcoholics, women on oral contraceptive pills or any other hormones, pregnant women and patients with clinical evidence of neuropathy, nephropathy, and retinopathy were not enrolled in the study. Twenty five (25) type 2 patients

and Twelve (12) type 1 cases of either sex gave their consent to participate in the study. Type 1 or 2 was decided based only on their age and their subsequent response to insulin and oral hypoglycemics, respectively. Thirty (30) individuals were chosen from attendants of the patients to serve as controls. All exclusion criteria of the test groups were applied to the control group also. The protocol was approved by the Institutional Ethics Committee.

Age, weight and height were recorded and body mass index (BMI) was calculated. Blood was collected as a random sample before the initiation of therapy in the diabetic patients and the following estimations were carried out:

1. *Random plasma glucose (RBS):* By the glucose oxidase method on Hitachi 917 autoanalyser using Roche Kits.

2. *Fibrinogen assay [10]:* Fibrinogen in plasma was converted to fibrin in the presence of calcium chloride. The fibrin clot was collected and digested with sodium hydroxide. Protein content of the clot was determined by the biuret method.

3. *Ceruloplasmin assay [11]:* At pH 5.4, ceruloplasmin catalyses the oxidation of paraphenylenediamine(PPD) to yield a coloured product which is believed to correspond either to Bandrowski's base or to Weuster's red. The rate of formation of the coloured oxidised product is proportional to the concentration of ceruloplasmin, if a correction is made for the nonenzymatic oxidation of PPD. Simultaneous estimations were carried out with and without sodium azide, which inhibits the nonenzymatic oxidation of PPD. The difference between the results of the two assays was proportional to the ceruloplasmin concentration.

4. *α-1 antitrypsin assay [12]:* The proteolytic enzyme trypsin hydrolyses casein, with the formation of smaller peptides. The enzyme reaction after suitable interval of time is arrested by the addition of trichloroacetic acid (TCA) which precipitates the proteins, but the peptides are soluble in the acid. The TCA soluble fragments are a measure of proteolytic activity of this enzyme. When the inhibitor is added to the preincubated mixture, it prevents the release of peptides by the proteolytic enzymes. Thus, the estimation of TCA soluble components in the presence and absence of inhibitor is a measure of inhibitory activity against proteolytic enzymes. The TCA soluble fragments were analysed by the method of Lowry *et al*. [13]. The final colour formed is a result of the reaction of the peptides with copper ions in alkali and reduction of the phosphomolybdic reagent by the presence of tyrosine and tryptophan present in the treated peptides.

5. *α-1 acid glycoprotein [14]:* After removing heat coaguable proteins with perchloric acid, the orosomucoid which remains in the solution was precipitated by phosphotungstic acid and estimated by determining its carbohydrate content by reaction with its tyrosine residues with folin ciocalteau reagent.

Statistics

The data was analysed by the students *t* test and the ANOVA test. Pearson's coefficient was applied for correlational analysis.

Results

The aim of the study was to examine inflammation as a pathogenetic cause in type 1 and type 2 freshly diagnosed diabetes mellitus cases.
The mean age (range), BMI and the number of males: females are presented in table 1. the control group participants were so chosen as to cover the age range of both the test groups.

Table 2 lists the values of random blood sugar (RBS) and acute phase proteins in the three groups as mean ± SD. Test groups T-1 and T- 2 had significant higher values of all the parameters in comparison with the control group. It is noteworthy that although T- 1 patients had very high RBS values as compared to the T-2 group, the levels of all the acute phase markers studied were higher in the T-2 patients.

This can be further appreciated in Table 3 which depicts the significance levels (p values) of the various groups.

Table 1: Patient Characteristics

	Type 1 (n = 12)	**Type 2** (n = 25)	**Controls** (n = 30)
Age	15.33 ± 4.6 (12-19 yrs)	48.28 ± 7.11 (32-60 yrs)	44.97± 15.06 (14-60 yrs)
BMI	19.49 ± 1.23	24.03 ± 1.46	21.75 ± 2.27
Males : Females	05 : 07	15 : 10	17 : 13

Table 2: Levels of the acute phase proteins as Mean ± SD

Parameters	Type 1 Mean ± SD	Type 2 Mean ± SD	Controls Mean ± SD
Random blood Sugar (RBS) (mg/dL)	338.25± 50.97	193.26±35.20	94.20±7.00
α1 antitrypsin (mg/dL)	495.70 ±32.77	562.16±63.00	350.48±114.07
α1 acid glycoprotein (mg/dL)	94.87±23.31	181.93±31.94	103.41±22.13
Ceruloplasmin (mg/dL)	40.69± 9.85	45.05±9.03	26.95±4.10
Fibrinogen (mg/dL)	434.65±46.36	572.25±82.26	335.34±42.19

Table 3: Significance (p value)

Parameters	T-1 v/s Controls	T-2 v/s Controls	T-1 v/s T-2
Random blood Sugar (RBS) (mg/dL)	< 0.0001	< 0.0001	< 0.0001
α1 antitrypsin (mg/dL)	0.0002	< 0.0001	0.003
α1 acidglycoprotein (mg/dL)	0.275*****	< 0.0001	< 0.0001
Ceruloplasmin (mg/dL)	< 0.0001	< 0.0001	0.190*****
Fibrinogen (mg/dL)	< 0.0001	< 0.0001	< 0.0001

$P \leq 0.05$ was considered significant.
*******Not significant*

Discussion

In the twelve newly diagnosed type 1 patients, the levels of α1- antitrypsin, ceruloplasmin and fibrinogen were found to be significantly increased as compared to the controls (Table 2). Previous reports on the acute phase reactants levels in type 1 diabetes are contradictory. While Crook MA *et al* [6]. have shown that serum sialic acid and acute phase proteins are not elevated in type 1 diabetes; Gomes *et al* [3] reported increased levels of CRP, α1-acid glycoprotein and fibrinogen in type 1 patients. Increased levels of fibrinogen, factor VII and whole blood viscosity was also found by John AD Elia *et al* [7] and Defeo *et al* [15]. We did not find any difference in the α1-acid glycoprotein levels. Twenty five (25) type 2 newly diagnosed patients showed increased levels of all the four proteins that were studied (Table 2). The findings are in agreement with most of the authors who worked with acute phase proteins in type 2 diabetes [4, 16,17]. The results show that the role of chronic low grade inflammation in the pathogenesis of type 2 diabetes seems possible beyond doubt. At the same time its role in type 1 diabetes cannot be completely ruled out.

The underlying mechanism for the augmented acute phase response is not well understood and the stimulus for this response is unknown. A number of hypothesis have been put forward to explain the activation of the inflammatory process and these include, insulin resistance, obesity, atherosclerosis, other diabetic complications and maladaptation of the normal innate immune response to environmental threats [18-20]. The most widely studied is the

association of obesity, insulin resistance, type 2 diabetes and acute phase reactants. It has been shown that adipocytes secrete a number of proinflammatory cytokines in the post-prandial state, [21-23], thus rendering obese individuals to higher chances of developing diabetes. Elevated glucose levels promote inflammation by increasing oxidative stress [24] due to the formation of advanced glycated end products (AGEs) and increased TNF (kappa) B. In this study, the mean BMI was found to be 19.49 ±1.23 in type 1 patients and 24.03 ±1.46 in type 2 patients. Correlational analysis did not establish any relation between BMI and the acute phase reactants ($r<0.3$). The values of the various parameters when compared between the untreated type 1 patients and type 2 patients reveal a significant increase in type 2 patients (Table 2&3). Even the ceruloplasmin values, although not statistically significant, were slightly higher in the type 2 patients. The mean random blood sugar (RBS) values in T-1 patients was 338.25 ± 50.97 mg/dL and that in T-2 cases was 193.26 ± 35.30 mg/dL. Inspite of this huge difference, the inflammatory markers levels were higher in the type 2 patients. No correlation was found between the degree of hyperglycemia and the levels of any of the acute phase proteins ($r < 0.3$). Hence the role of glycemic status in the activation of inflammation is not very satisfactory. Evidence is also available to say that inflammatory markers are elevated well before the clinical manifestation of hyperglycemia [25]. This again gives credence to the findings that the degree of inflammation may not be on par with the extent of hyperglycemia. Since our study was in newly diagnosed patients, it is convincing that the inflammatory process begins much earlier than the clinical manifestation and laboratory evidence of diabetes. However, research has shown that decreasing plasma glucose levels decreases the concentration of the acute phase reactants [26]. Also, 2 hrs postload, glucose values showed positive correlation with the inflammatory markers in a report on women with previous history of gestational diabetes [27].

The course of the disease and the resulting complications in either type 1 or type 2 diabetes remains the same, particularly, the atherosclerotic risk. Fibrinogen is identified as an independent risk factor in the development of ischemic heart diseases [28]. Barrazzani R *et al* [29] found a suppression of fibrinogen production when insulin was infused to nondiabetics and patients with type 1 diabetes; but the same in type 2 patients showed an increased production of fibrinogen. Our type 1 patients were freshly diagnosed cases and the study was conducted before the initiation of insulin therapy in them. Fibrinogen levels were significantly higher in both groups ($p<.0001$) in comparison with the controls. The difference in the values between T-1 and T-2 groups also reached high significance ($p<.0001$). Barazzoni *et al* [29] postulated that an altered response to insulin causes hyperfibrinogenemia in type 2 diabetics. Insulin is an inhibitor of the acute phase response, exerting its effect at the transcriptional level, thereby preventing the expression of the particular protein gene [30]. Why then do the type 2 patients with generally higher insulin levels have a more severe acute phase response? The answer probably lies in the resistance to the action of insulin by the insulin-sensitive tissues.

Ceruloplasmin has for long enjoyed the status of an antioxidant [31]. Eduardo Ehrenwald [32] showed a very interesting feature of ceruloplasmin. The intact human ceruloplasmin which is a 132 KD molecule caused increased oxidation of LDL *in vitro*. Starkebaum and Harlan [33] also showed that an increased serum ceruloplasmin could result in excess oxidized LDL, and causes vascular injury by generating free radicals such as hydrogen peroxide. High levels of ceruloplasmin were found in both types of diabetics. This also partly explains the atherosclerosis risk in diabetes. Further work needs to be undertaken to establish the role of ceruloplasmin in coronary artery diseases.

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