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Circulating Immune Complexes and Microvascular Complications in I.D.D.M.

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Abstract

Microvascular complications occurring in diabetes mellitus may be attributed to the long standing metabolic derangement, platelet abnormalities or to circulating immune complexes present in the serum of patients especially those with insulin-dependent diabetes mellitus. Twenty patients with insulin dependant diabetes mellitus (IDDM) and 10 age-matched controls were subjected to a study of their ADP-induced platelet aggregation. Detection of IgG aggregates and/or circulating immune complexes (CIC) has been done using an indirect ELISA technique. Significantly higher serum levels of IgG aggregates and/or CIC were found in IDDM patients compared to normal controls. Elevated levels were detected in 65% of patients. A significant positive correlation was found between fasting blood sugar and IgG aggregates and/or CIC. Hence measurement of CIC in IDDM may serve as a predictive test for the development of microangiopathic complications in IDDM.

Introduction

TYPE I (IDDM)diabetes mellitus is a disorder in which the damage of the insulin-producing beta cells of the pancreatic islets of Langerhans results in a deficiency of insulin. Although it has only recently been associated with an immune pathogenesis, it is now clear that there is an autoimmune cause in the great majority of patients with IDDM [1].

Vascular complications have accounted for the majority of the morbidity and mortality in patients with diabetes [2].

Some studies have documented that platelet abnormalities occurred in diabetes [3], and may play a role in the pathogenesis

of the microangiopathy in IDDM. Circulating immune complexes have been detected in the plasma of patients with IDDM and it has been suggested that they may participate in the pathogenesis of platelet abnormalities, leading to microvascular complications [4].

The aim of this work was to study the level of IgG aggregates and circulating immune complexes in the serum of patients with IDDM and to try to detect a possible correlation between such findings and the degree of platelet dysfunction if present..

Material and Methods

Twenty patients with IDDM, whose ages ranged from 6 years - 18 years were chosen from attendants of the endocrine clinics of the New Paediatrics Hospital and the sections of Internal Medicine of Kasr El Aini Hospitals.

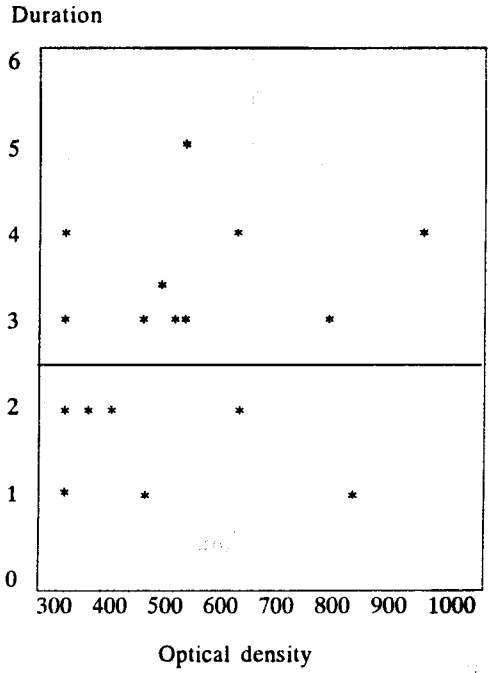
Ten age-matched controls were also enrolled in the study. Patients and controls were subjected to the following:

1. Complete blood and platelet counts.
2. Fasting blood sugar level.
3. ADP-induced platelet aggregation.
4. Specific laboratory tests for detection of IgG aggregates and CIC using an indirect ELISA technique [5]. Alkaline phosphatase-conjugated antihuman IgG (gama-chain specific-Sigma product No A 3150) was utilized to determine the O. D. of the reaction, after subjecting it to a substrate of p-nitrophenyl phosphate (Sigma product N. N. 2765).

The O. D. of the test serum was calculated by subtracting the mean O. D. of the normal control sera from that obtained with the test serum. An O. D. 25D above the mean of the normal controls was considered positive.

Results

The results are shown in table (1) and figures (1&2).



r = 0.0139
No correlation was detected

Fig. (1): Correlation between duration and optical density

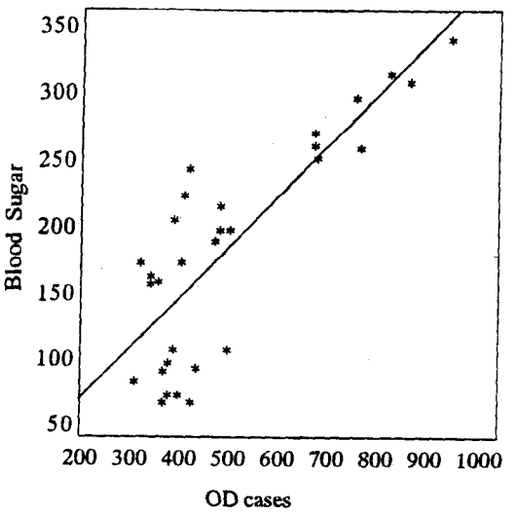


Fig. (1): Curve showing significant direct linear correlation between fasting blood sugar and OD values of ELISA for the detection of antibodies.

Discussion

Increasing evidence that autoimmune mechanisms play a role in IDDM is now being gathered [6]. The postulated sequence of events leading to islet cell destruction starts by an initiating event such as a viral infection, with a resulting inflammatory response to beta cells of the islets. This inflammation is characterized by HLA-DR expression on the beta cells and lymphocytic infiltration of islets. Subsequently, either a persistent stimulation of the immune system or a defect immune regulation allows the propagation of the autoimmune response in a genetically predisposed individual. This causes destruction of the beta cells and leads to insulin deficiency [1].

Table (1): Statistical Analysis of Patient and Control Groups.

No. of cases	Control 10	Patient 20	*p* value	Statistical significant
Platelet count X 10^3/ cumm	272.8 + 44.284	299.6 + 70.29	0.00721	N.S. N.S.
O.D. values for ELISA	369.1 + 47.377	556.35 + 191.605	0.0001	H.S.
No. of +ve cases	1/10 (10%)	13/20 (65%)		
Platelet aggregation %	77.2 + 10.748	71.15 + 12.171	0.19936	N.S.
Blood sugar mg/ dl	86.8 + 10.554	225.75 + 56.699	0.0001	H.S.

N.S. = Non significant
H.S. = Highly significant

Evidence of autoimmune mechanisms include an increased association with autoimmune reactions against other organs, the presence of serum antibody against islet cells that may interfere with the in vitro function of such cells and the presence of serum anti-insulin antibodies, even before insulin treatment starts. An immunoglobulin in the serum of some patients with insulin-resistant diabetes appears to inhibit binding of insulin to its receptor [7].

The long-standing metabolic derangement occurring in diabetes is frequently associated with permanent and irreversible functional and structural changes in the cells of the body, those of the vascular system being particularly susceptible [8].

Such vascular complications have accounted for the majority of the morbidity and mortality in diabetics.

A number of studies have suggested involvement of the haemostatic system in the initiation and propagation of the vascular lesions and platelet abnormalities have been documented in diabetics [3].

These include increased adhesiveness, increased in vitro aggregation and increased availability of PF_3 [9].

Circulating immune complexes resulting from binding of auto-antibodies to their respective antigens, whether anti-B-islet cell antibodies or anti-insulin antibodies have been implicated in the pathogenesis of the microvascular complications of IDDM [4].

In the present study, 20 patients with IDDM and 10 age-matched controls were subjected to a study of their serum level of CIC and IgG fixed to normal donor platelets by using an indirect ELISA technique [5]. Correlation studies between such findings and other parameters as platelet counts, ADP-induced platelet aggregation and fasting blood sugar levels were attempted. An increased O. D. Value for ELISA was detected in 13/20 patients (representing 65%). Such patients were considered positive for the presence of IgG and CIC in the test sample which bound to normal platelets. This agrees with the results demonstrated by Virella et al [4] who found a high level of soluble immune complexes in 7 out of 8 diabetic patients.

In another study performed by Triolo [10], he reported that 30% of his patients had increased amounts of PA IgG. According to his view, this IgG represented in vivo platelet bound circulating immune complexes in patients with IDDM.

With regards to platelet aggregation studies, no significant differences in the results were found between patient and control groups (Table 1). In coordinance with our work Alessandrini et al [11] reported normal platelet aggregation in non complicated cases, of IDDM. However, Di Minno et al [12] found increased platelet aggregation in their study group. Triolo et

al [5]. reported that increased PA IgG was associated with a high degree of platelet aggregation, in their study performed on a group of patients with IDDM as compared with a group of age-matched controls. However, our findings showed no significant correlation between O. D. values and platelet aggregation results.

Interestingly, a highly significant positive correlation ($p < 0.01$) (table 1 - fig.2) was found between fasting blood sugar level and OD values of ELISA tests, although these patients already established long-standing diabetics (mean duration was 2.675 + 1.195 years). This may be explained by the increased doses of insulin given to such patients with increasing blood sugar levels, leading to formation of insulin-anti-insulin immune complexes. Such circulating immune complexes bind to platelets their Fc receptors.

Triolo [5] suggested that thee complexes may contribute to the development of microangiopathy by increasing platelet aggregation inducing the release of vasoactive amines. This increases vascular permeability and allows such complexes to be deposited in or around the vessel wall with the subsequent development of inflammatory and destructive lesion.

In conclusion, we suggest that the application of such an assay for detection of PA IgG and CIC, either directly or indirectly, may act as a predictive parameter for the development of microvascular complications in IDDM.

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