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Study of Humeral Immunity and Complement Activity in Diabetes Mellitus in Vidarbha Region of Maharashtra

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Abstract

***Background**: Diabetes mellitus is the most common of the serious metabolic diseases. Diabetes patient is susceptible to a series of complications that cause morbidity and premature mortality. Several aspects of immunity are altered in patients with diabetes mellitus, polymorphonuclear leukocyte function is depressed. The clinical data on humoral immunity is limited. **Materials and Methods**: This study comprises of 305 patients of diabetes mellitus with or without soft tissue infections attending or admitted to Government Medical College Nagpur. Presence of any metabolic complications of diabetes mellitus like, diabetic ketoacidosis/ketonuria, diabetic hyperosmolar coma were excluded from the study. **Results**: There was increase in IgG level in diabetes mellitus patients i.e. 2146.11 mg/dl as compared to control (1525 mg/dl) also increase in Ig A level in diabetes mellitus patients (307.07 mg/dl) as compared to control (158 mg/dl) and IgM in diabetes mellitus patients (190.32 mg/dl) as compared to control (150 mg/dl). But there was decrease in level of C3 in diabetes mellitus patient (97 mg/dl) compared to control (120 mg/dl). All these differences were statistically significant. IgG, IgA IgM an C3 in patients of diabetes mellitus with or without infections and associated factors shows no difference in level. **Conclusion:** There is significant raise in IgG, IgA, IgM levels indicating overall stimulation of humoral immune system in diabetes mellitus and significant depletion of complement component C3.*

***Keywords**: Diabetes mellitus, single radial immunodiffusion, humoral immunity*

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Introduction

Humoral immunity depends upon the appearance in the blood of globulins known as antibodies or immunoglobulins, a product of B-lymphocytes and their precursors. These combines specifically with antigen of the kind that specifically stimulates its production and this union can lead to some remarkable reactions like agglutination, neutralization, phagocytosis, lysis of bacteria and RBCs as result of activation of complement. Humoral immunity in addition to its own function forms a link between the phagocytic function and cell mediated immunity. [1,2]

In Cell Mediated Immunity, T-lymphocyte (thymus dependent) plays a key role in the mediation of this type of immunity by way of production of lymphokines which are non-antibody soluble substance produced by lymphocytes of immunologically competent individual an antigenic stimulation. Lymphocyte mediators might relate to the development of cellular immune response in the following way. Antigen sensitive lymphocyte when stimulated by the appropriate antigen becomes activated and start synthesizing the various lymphocyte mediators, chemotactic factors for monocyte and polymorphonuclear leukocyte to recurrent inflammatory cells to the reaction site once there, the polymorphonuclear and macrophage

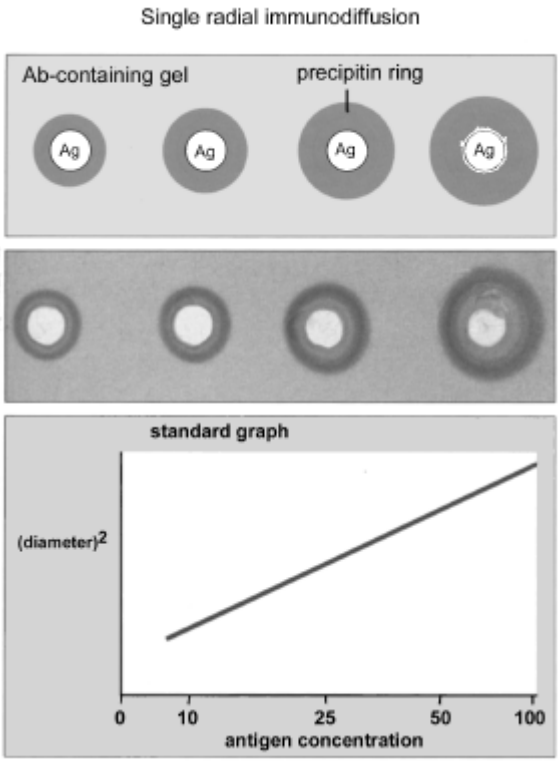
might be activated to enhanced state by activating factors. Other lymphocytes are recruited to participate in the reaction by mitogenic factor. These events have the effect of amplifying an initially small reaction once activated, the inflammatory cells become bactericidal or tumoricidal. Furthermore, the vasoactive properties of some of mediators may account for the part of inflammation. Other protein systems including the complement system has kinin system and the clotting system are also called in to plays. In addition, there may be control system which inactivate the mediators as they are produced. Immunoglobulins, which in addition to its own functions like agglutination, neutralization etc. forms a link between phagocyte function and cell mediated immunity. [3]

The term complement (C) refers to a system of factors which occurs in normal serum and are activated characteristically by antigen antibody interaction and subsequently mediate a number of biologically significant consequences. Complement, ordinarily does not bind to free antigen or antibody but only to antibody, which has combined with its antigen. Complement system consisted of at least 20 chemically and immunologically distinct serum proteins comprising the complement components, the propardin system and the control proteins. [4] In the present study, humeral immunity in Diabetes Mellitus patients with or without soft tissue infections associated with insulin therapy, Hypertension, Hyperglycemia, and Duration of Illness was seen.

Materials and Methods

This study comprises of 305 patients of Diabetes Mellitus with or without soft tissue infections attending or admitted to Government Medical College and Hospital, Nagpur for two years. Presence of any metabolic complications of diabetes mellitus like diabetic ketoacidosis/ketonuria, diabetic hyperosmolar coma were excluded from this study.

These patients were thoroughly evaluated and investigated to determine presence of complications like retinopathy, neuropathy, nephropathy, coronary artery disease, peripheral vascular disease, cardiovascular events etc. The findings in each patient were recorded as per local and systemic examination. Quantitation of serum immunoglobulins, IgG, IgA & IgM and serum compliment C3 components was done by using single Radial Immunodiffusion. [5] 3.5 ml of venous blood was collected aseptically from the antecubital vein from each patient in sterile plain bulb. It was allowed to clot and serum was separated after centrifugation. Sodium azide was added to it as a preservative. Serum samples were stored at 4°C till the immunological investigations were done.



Results

Three hundred and five patients of diabetes of mellitus with and without soft tissue infections were recruited in this study. Out of 305 patients of diabetes mellitus and 203 (66.55%) patients were without soft tissue infections and 102 (33.45%) were with soft tissue infections. 220 (72.15%) of these were males and 85 (27.85%) were females. The male to female ratio was 2.6 to 1. The mean age of patients was 49.66.

Out of 102 patients of diabetes mellitus with infections, 82 (80.40%) were male and 20 (19.60%) were females. The mean age in this study was 50.22, the mean age of male patients was 50.32 (11.58%) and the mean age of female patients was 43.81(11.89%). Maximum number of patients belonged to age group 41-50 i.e.32

(31.37%) and 28 (27.45%) in age group 51-60 Yrs.

Out of 203 patients of Diabetes mellitus without soft tissue infection 138 (67.98%) were males, and 65 (32.02 %) were females. The mean age in this study was 49.11 (13.58%), the mean age of male patients was 49.11 (13.58%), and the mean age of female patients was 47.03 (10.74%) Majority of patients belonged to age group 41-50 i.e 70 (34.48%) followed by 56 (27.58%) in age group above 60 years.

There was increase in IgG level in diabetes mellitus patients i.e. 2146.11 mg/dl as compared to control (1525 mg/dl) also increase in Ig A level in diabetes mellitus patients (307.07 mg/dl) as compared to control (158 mg/dl) and IgM in diabetes mellitus patients (190.32 mg/dl) as compared to control (150 mg/dl). But there was decrease in level of C3 in diabetes mellitus patient (97 mg/dl) compared to control (120 mg/dl). All these differences were statistically significant (Table- 1).

There were no differences in levels of IgG (2071.35 mg/dl), IgA (304.64 mg/dl), IgM (177.01 mg/dl) and C3 (99.64 mg/dl) in patients without infection as compared to IgG (2294.9 mg/dl) IgA (311.09 mg/dl) IgM (157 mg/dl) and C3 (91.75 mg/dl). All these differences were not statistically significant (Table- 2).

There were no statistically significant differences in IgG, IgM, IgA and C3 level in patient which were on oral therapy and insulin therapy. Same with patients which were hypoglycaemia and normoglycaemia, hypertensive and normotensive, duration of illness upto 5 years and duration of illness above 5 years (Table- 3,4,5,6).

Table- 1: Mean immunoglobulins IgG, IgA, IgM & complement C3

Immunoglobulins and complement C3	Patients (305) (mg/dl)	Control (100) (mg/dl)
IgG	2146.11	1525.69*
IgA	307.07	158*
IgM	170.32	150*
C3	97	120*

**= p value less than 0.0001*

Table- 2: Mean values with or without infections

Immunoglobulins and complement C3	Without infection (mg/dl)	With infection (mg/dl)
IgG	2071.35	2294.9
IgA	304.64	311.9
IgM	177.01	157
C3	99.64	91.75

p value more than 0.05

Table- 3: Mean values in treatment groups

Immunoglobulins and complement C3	Treatment	
	Oral	Insulin
IgG (mg/dl)	2062.04	2246.51
IgA (mg/dl)	305.66	309.33
IgM (mg/dl)	174.81	169.38
C3 (mg/dl)	95.25	91.84

p value more than 0.05

Table- 4: Mean values and blood sugar

Immunoglobulins and complement C3	Blood Sugar	
	Normal	Raised
IgG (mg/dl)	2209.96	2223.35
IgA (mg/dl)	304.19	305.47
IgM (mg/dl)	178.86	179.07
C3 (mg/dl)	94.04	94.52

p value more than 0.05

Table- 5: Mean values and duration of illness

Immunoglobulins and complement C3	Duration of illness	
	< 5 years	> 5 years
IgG (mg/dl)	2164.73	2037.6
IgA (mg/dl)	306.5	330.21
IgM (mg/dl)	170.34	169.31
C3 (mg/dl)	96.17	95.79

p value more than 0.05

Table- 6: Mean values and blood pressure

Immunoglobulins and complement C3	Blood Pressure	
	Normal	Raised
IgG (mg/dl)	2184.62	2149
IgA (mg/dl)	302.29	301.23
IgM (mg/dl)	167.83	166.95
C3 (mg/dl)	95.95	96.01

In diabetes patients the mean serum immunoglobulin levels IgG (2146.11 mg/dl) IgA (307.07 mg/dl) and IgM (170.32 Mg/dl) as compared to healthy controls (IgG: 1325.69 mg/dl, IgA, 138 mg/dl IgM: 150 mg/dl) were significantly raised ($P<0.05$); while serum complement component C3 (97.0 mg/dl) were

significantly (p<0.05) reduced as compared to healthy controls (120 mg/dl) table- 1. However, there was no significant difference between patients with infection (IgG : 9 mg/dl, IgA: 311.9 mg/dl, IgM: 157 mg/dl, C3: 9-75 mg/dl) and patients without infection (IgG: 2071.35 mg/dl, IgA: 304.64 mg/dl, IgM: 177.01 mg/dl, C3: 99.64 mg/dl) table-2, Similarly there was no significant difference between patients on oral therapy (IgG 2062.04 mg/dl, IgA: 305.66 mg/dl, Ig: 174.81 mg/dl, C3: 95.25 mg/dl) and patients on insulin therapy (IgG: 2296-55 mg/dl, IgA: 309.33 mg/dl, IgM: 169.38 mg/dl, C3: 91.84 mg/dl) table-3. There was no significant difference between patients with normal blood sugar cases (Table- 4). There was no significant difference between patients with normal blood pressure and pressure with raised blood pressure (Table- 6). Also, no significant difference was observed in the immunoglobulin levels and C3 levels in patients with less than 5 years duration and in patients with more than 5 years duration of diabetes (Table- 5).

In the present study, the serum IgG, IgA and IgM levels were significantly raised in patients of diabetes mellitus as compared to healthy controls. It indicates the generalized stimulation of humoral immune system. However, no significant difference was observed along patients with infections and without infections. Similarly, there was no significant difference of immunoglobulins levels in presence or absence with various associated factor like insulin therapy, raised blood sugar, raised blood pressure and increased duration of diabetes mellitus.

Discussion

Diabetes mellitus is chronic metabolic disease known to man since ages. The incidence of diabetes mellitus increases usually after 30 years and these patients are prone to develop complication like microangiopathy e.g. Retinopathy, Nephropathy, Neuropathy etc. and conditions like Coronary Artery Disease, Peripheral Vascular Disease, Cerebrovascular Events, Soft Tissue Infections etc. Early detection of occurrence of complications and prompt intervention at this stage may reduce morbidity and mortality to a great extent.

Patients with diabetes mellitus have more infections than those of without diabetes. One of the possible causes of this increased prevalence of infections is defect in immunity. Besides some decreased cellular responses in vitro, no disturbances in adaptive immunity is seen in diabetic patients. Different disturbances that complement factor 4 decreased cytokine response after stimulation in humoral innate immunity. Geerlings S. E. et al; [6] described the immune dysfunction with diabetes mellitus. Their study showed decreased functions (chemotaxis, phagocytosis, killing) of diabetic polymorphonuclear cells and diabetic monocytes/macrophages compared to cells of controls. In general, a better regulation of the diabetes mellitus leads to an improvement of these cellular functions. Furthermore, some microorganisms become more virulent in a high glucose environment. Patients with diabetes mellitus have infections more often than those without diabetes mellitus. One of the causes of this prevalence of infection is defect in immunity. Geelings S. E. et al; [6] described the disturbances in adaptive immunity in diabetic patient. Different disturbances (low complement component 4, decreased cytokine response after stimulation) in humoral innate immunity. Zemlaikara Z. M. & Kravets B. B. [7] studied on activity of nonspecific protection factor (complement titer, lysosome level, complement, phagocytoses index and serum immunoglobulins (IG) C.D) in 70 diabetes mellitus patients. Cheta D et al; [8] studied immunoglobulin A in both type I and type II diabetes there was no significant difference. In the present study, the serum IgG, IgA and IgM levels were significantly raised in patients of diabetes mellitus as compared to healthy controls. It indicates the generalized stimulation of humoral immune system. However, no significant difference was observed along patients with infections and without infections. Similarly, there was no significant difference of immunoglobulin's levels in presence or absence with various associated factor like insulin therapy, raised blood sugar, raised blood pressure and increased duration of diabetes mellitus. Blackwell CC et al; [9] found that there were lower levels of C3 and C4 components of the complement system in patients with insulin diabetes mellitus (IDDM) but not among those with non-insulin dependent diabetes mellitus (NIDDM). Krantz S. et al [10] found slightly

elevated C3 levels in blood plasma of both types of diabetes. Morimoto Y et al; [11] suggested that there is a high level of complement in both types of diabetes mellitus but the complement activation seems to be much enhanced in IDDM compared to NIDDM. Smith W. L. et al [12] described increased prevalence O ($P < 0.001$) of IgA deficiency in juvenile onset IDDM but not in adults IDDM. Glycemic control somewhat plays role immune dysfunction in patients with diabetes mellitus. Geerlings S. E. et al; [6] suggested that better regulation of diabetes mellitus leads to an improvement of these cellular functions furthermore, some microorganisms become more virulent in high glucose environment possibly the carbohydrate composition of receptor plays a role in this mechanism. In the present study, the compliment component C3 levels in diabetes patients were significantly less than the healthy controls. This is contradictory to the observation of Krantz et al; [10], Morimoto Y et al; [11] and similar to Blackwell C. C. et al; [12]. The insulin containing circulating immune complexes have been reported. The lower levels of C3 may be ascribed to the formation of immune complexes that occurs in diabetes mellitus.

The result of the humeral immune system in the present study indicates that immunoglobulin's levels IgG, IgA and IgM are raised with depletion of C3 level. However, level shows no significant deviation in presence of infections or other factors. It denotes that although there is a generalized stimulation of immune system, but higher predisposition of infection especially increased severity as seen in diabetes could be due to association of other factors like phagocytic dysfunction, decrease chemotaxis etc. or due to non-immune factors like microangiopathy in the vessels of limbs, polyneuropathy.

Conclusion

Bacterial isolates are generally multidrug resistant and resistance must be considered for proper antibiotic therapy. There is significant raise in IgG, IgA, IgM levels indicating overall stimulation of humeral immune system in diabetes mellitus and significant depletion of complement component C3. No significant difference in immunoglobulin IgG, IgA, IgM and C3 level in patients with and without infections and other associated factors observed by us.

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