

# Circulating immune complexes, immunoglobulin classes (IgG, IgA and IgM) and complement components (C3c, C4 and Factor B) in diabetic Nigerians.

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## Summary

**Objective:-** To evaluate serum levels of circulating immune complexes (CICs), immunoglobulin classes (IgG, IgA and IgM) and Complement Components (C3c, C4 and Factor B) in Nigerians with Type 1 or Type 2 diabetes mellitus.

**Design:-** Case control study.

**Setting:-** University College Hospital, Ibadan, Oyo State, Nigeria.

**Subjects:-** Forty-two subjects with diabetic mellitus (17 Type 1 D. M. and 25 Type 2 D. M.) and 21 apparently healthy control subjects.

**Intervention:-** Serum level of CICs was measured by polyethylene glycol precipitation method while single radial immunodiffusion method was used to measure serum levels of immunoglobulins and complement components.

**Results:-** Only CICs were significantly higher in Type 1 diabetic subjects compared with the controls whereas CICs C3c, C4 and IgM were significantly increased in Type 2 diabetic subjects compared with the controls. The levels of CICs, C3c and IgM were significantly elevated in Type 2 diabetics compared with Type 1 diabetics.

**Conclusion:-** CIC concentrations may serve as a useful index of depressed host defences usually associated with diabetes mellitus and that humoral immunity is deranged more in Type 2 diabetics compared with Type 1 diabetics. Probably as a result of hyperinsulinaemia associated with insulin resistance.

**Keywords:** Immune complexes, Immuno-globulins, Complement factor, Diabetic mellitus, Deranged..

## Résumé

**Objectif:-** Evaluer le taux du sérum de la circulation des types d'immunoglobuline complexe immune (IgG, IgA, et IgM) et composant du complément (C3c, C4 et Bfacteur) chez les Nigeriens avec type 1 or type 2 diabète mellitus.

**Plan:-** Une étude de cas d'un contrôle.

**Cadre:-** Collège hospitalier universitaire, Ibadan, Etat d'Oyo, Nigeria.

**Sujets:-** Quarante deux sujets atteints du diabète mellitus (17 type 1 D. M. et 25 Type 2 D. M.) et 21 apparemment en très bonne santé sujets de contrôle.

**Intervention:-** Taux de sérum de CICs était mesuré par la méthode de la précipitation glycol polyéthylène tandis que la méthode d'immunodiffusion seul radial était utilisée pour mesurer le taux de sérum de taux de l'immunoglobuline et composants du complément.

**Résultats:-** CICs seulement était remarquablement élevé chez les sujets diabétiques Type 1 par rapport au contrôle tandis que C3c, C4 et IgM étaient remarquablement élevés chez des sujets diabétiques Type 2 par rapport aux contrôles.

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Les taux de CICs, C3c et IgM étaient remarquablement élevés chez les diabétique type 2 par rapport aux diabétiques Type 1.

**Conclusion:-** La concentration de CIC pourrait servir comme un indice très utile comme des défences pour des hôtes déprimés le plus souvent, associé avec diabétiques mellitus et que l'immunité à médiation humorale (par voie sanguine), est de plus dérangée chez les diabétiques Type 2 par rapport aux diabétiques type 1. Peut-être attributable à l'hyperinsulinémie associée avec la résistance d'insuline.

## Introduction

The fact that diabetics are more susceptible to infections than the non-diabetic (healthy) population suggests that immunologic capability may be deranged in the diabetic population.<sup>1,2</sup> In both Type 1 and Type 2 diabetics, neutrophil chemotaxis, phagocytosis and adhesion were found to be impaired.<sup>2</sup> Garagen<sup>3</sup> described abnormal number of T-lymphocytes, B-lymphocytes and T-lymphocyte subsets in Type 1 diabetics.

In diabetes mellitus, hyperglycaemia results from a defect in insulin secretion or action or both. Type 1 diabetes is mainly associated with auto-immune destruction of insulin producing of the islet cells of the pancreas.<sup>4,5</sup> Moreover, in Type 1 diabetics, complement fixing islet cell antibodies were detected before overt signs of pancreatic deficiency were apparent.<sup>6</sup>

Type 2 diabetics is mainly due to insulin resistance.<sup>6</sup> Compared with controls, serum IgM and IgA were found to be elevated in Type 2 diabetics' whereas IgG and IgM were elevated in Type 1 diabetics.<sup>8</sup> These differences in immunoglobulin classes found in sera of Type 1 and Type 2 diabetic subjects may explain differences in the characteristics mouth flora of Type 2 compared with Type 1 diabetics with periodontitis.<sup>2</sup> This suggests differential immune responses in Type 1 and Type 2 diabetes; therefore further analysis of humoral immune parameters in diabetic subjects is required.

## Materials and methods

Forty-two subjects with diabetes mellitus randomly selected from the diabetic clinic at the Medical Outpatient Department of the University College Hospital, Ibadan, Nigeria were studied. Diabetes mellitus was defined according to WHO criteria.<sup>9</sup> Twenty-five ages matched healthy individuals comprising mainly of hospital staff served as controls. Ten (10cm<sup>3</sup>) of blood was collected from each subject with 5cm<sup>3</sup> each put into both plain and fluoride oxalate bottles to obtain serum and plasma samples respectively. These were stored at -20°C until analyzed. Immune complexes were determined immediately using polyethylene glycol (PEG) precipitation method described by Haskova et al.<sup>10</sup> Serum sample was diluted 1 in

3 with borate buffer pH 8.4, 0.22cm<sup>3</sup> of the diluted serum was added to 2.0cm<sup>3</sup> of 4% PEG 6000 solution and mixed thoroughly. For each diluted serum, a blank was set up by mixing 2.0cm<sup>3</sup> of borate buffer with 0.22cm<sup>3</sup> of the diluted serum. Incubation was at room temperature for 1 hour. The optical densities were read at 450nm in a spectrophotometer against serum blank. This was read off a standard immune complex calibration curve.

IgG, IgA, IgM, C3c, C4 and FB levels were measured by the single radial immunodiffusion method of Fahey and Mckelvey<sup>11</sup> as modified by Salimonu et al.<sup>12</sup> Commercial mono-specific antisera to these human protein (Serotec, Oxford, England) were used. The levels of IgG, IgA, IgM, C3c, C4 and FB were measured against commercial serum standards (Behringwerke AG, Marburg, Germany). Wells of equal diameter were cut in the antibody/agar mixture. The wells were filled with test or standard serum. The diameters of the precipitin rings were measured with a Hyland viewer after incubation for a maximum of 18 hours. Whole blood glucose levels was determined based on a method previously described by the WHO.<sup>9</sup>

## Results

The diabetic patients aged between 41 - 60yrs while that of the controls was between 40 - 65yrs. All results are shown in Table 1. The mean level of fasting - and 2 hrs postprandial

**Table 1 Blood glucose levels and humoral immunological parameters in diabetic patients compared with the controls.**

Parameters	Controls n = 21	Type 1 D.M n = 17	Type 2 D. M. n = 25
FBG (mg/dl)	63.1 ± 20.9	170.4 ± 131.9*	182.3 ± 131.1*
2hrs PPBG (mg/dl)	82.6 ± 23.0	199.0 ± 68.6*	264.4 ± 152.2*
CICs (mg/dl)	8.73 ± 10.8	15.2 ± 10.0*	19.0 ± 10.1*•
C3c (mg/dl)	84.5 ± 18.3	71.8 ± 20.4*	100.2 ± 26.7*•
C4 (mg/dl)	27.4 ± 10.5	29.8 ± 12.4	36.4 ± 18.2*
FB (mg/dl)	0.12 ± 0.03	0.14 ± 0.04	0.16 ± 0.07
IgG (mg/dl)	2211 ± 932	2450 ± 548.9	2552 ± 637.3
IgA (mg/dl)	301 ± 142	328 ± 85.8	326 ± 110.5
IgM (mg/dl)	440 ± 221	413 ± 221	708 ± 255*•

n = Number

FBG = Fasting blood glucose

2hrs PPBG = 2 hrs post prandial blood glucose

CICs = Circulating immune Complexes

\* = Significantly different from the controls (p < 0.05)

• = Significantly different from Type 1 (p < 0.05)

whole blood glucose were significantly higher in diabetic subjects than in control individuals. The glycaemic control in both types of diabetic was poor. The levels of CICs were also significantly elevated in diabetic Nigerians compared with their non-diabetic counterparts. The levels of CICs in Type 2 were significantly higher than in Type 1 diabetic subjects.

IgG and IgA levels were not significantly different between the three groups of subjects. Mean IgM level was significantly higher in Type 2 diabetics compared with controls or Type 1 diabetics. Mean C3c level was higher in Type 2 diabetics compared with either Type 1 subjects or the controls while C4 was significant higher in Type 2 subjects compared with controls. FB levels were similar in Type 1 diabetes, Type 2 diabetes and the controls.

## Discussion

Significantly higher mean level of CICs has been observed in Nigerians with diabetes compared with healthy non-diabetic controls in this study. Other studies have documented the presence of CICs in the sera of newly diagnosed insulin - dependent (Type 1) and diabetics. The presence of CICs has been observed to correlate with anti-islet cell antibodies in Type 1 diabetic subjects or a wide range of antiviral antibodies or HLA phenotype.<sup>13</sup> This might suggest the involvement of anti-islet cell autoantibodies in the pathogenesis of Type 1 diabetes mellitus. Elevated levels of CICs could then result from islet cell related antigen- autoantibody reactions. Immune complexes do act as serum blockers of immune system.<sup>10</sup> It can also inhibit blast transformation of lymphocytes in response to antigens.<sup>15</sup> Raised levels of CICs in these diabetic Nigerians (particularly Type 2 subjects) may impede effective immune response to pathogens, thus making the diabetics susceptible to infectious agents. Elevated CICs were found in poorly controlled diabetics, uncontrolled diabetics and diabetics with complications.<sup>16,17</sup> Based on this it is expected that CICs in our diabetic subjects were high as they were poorly controlled, thus supporting earlier reports. It is however, surprising that CICs levels were higher in Type 2 diabetes whose pathogenesis does not involve anti islet cell antibodies.

Factor B is a component of alternative pathway (APW) of complement activation while C4 is useful during classical pathway (CPW) of complement activation. CPW inhibits the formation of precipitation immune complexes in the plasma while APW solubilizes immune complexes that have already precipitated.<sup>22</sup> Elevated level of C4 in Type 2 diabetic individuals compared with the controls may be a result of increased C4 synthesis. The implication of elevated C4 in Type 2 diabetics is reduced formation of precipitating immune complexes. Precipitating immune complexes are solubilized by APW, whose products are present in blood as part of CICs.<sup>22</sup>

The most abundant factor in complement system is C3 which is involved in both CPW and APW. It produces C3a, C3b, C3bBb and C4C2aC3b which are potent neutrophil chemoattractment, opsonin and C5 convertases respectively. C3b is inhibited by factor H and factor I to produce C3c and C3dg,<sup>22</sup> therefore a higher level of C3c in Type 2 diabetics compared with Type 1 or controls shows that opsonisation and initiation of membrane attack complex may be affected in Type 2 diabetics.

Comparable levels of IgG and IgA were observed in diabetics and the controls. Previous studies produced conflicting results on immunoglobulins and complement factors in diabetics. Raised levels of IgA, C4, FB, IgG and IgM<sup>1, 8,18</sup> reduced level of immunoglobulin classes, normal levels of immunoglobulin classes and complement components<sup>20</sup> were reported in both types of diabetics compared with the controls. Type 2 diabetes is due to a complex biochemical disturbances of insulin in action, insulin resistance or both.<sup>7</sup> Some Type 2 diabetic subjects are hyperinsulinaemic together with high serum glycosylated proteins/lipoproteins.<sup>7</sup> Free insulin and glycosylated proteins have been found to be immunogenic,<sup>21</sup> it is therefore not

surprising to detect raised levels of IgM (a natural antibody) in Type 2 diabetics compared with the controls or Type 1 diabetics.

The finding of high levels of CICs in diabetic subjects (particularly Type 2) provide an additional justification for deranged immunity in diabetes patients. Other host defense mechanisms such as opsonisation and complement haemolytic activities (AH50 and CH50) need to be investigated in Nigerian diabetics.

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