region of IgG are impaired. These include alteration in the binding of proteins and fixation of complement to the FC fragment, which probably contributes to the increased susceptibility to infections [5] in these subjects. The increased incidence of infections in patients with diabetes mellitus (DM) is well documented in literature and some of these infections are also more likely to have a complicated course [6], [7]. In a previous study by Derensinski, diabetic ketoacidosis was complicated by an infection in 75% of the cases. Possible causes include defects in immunity, increased adherence of microorganisms to diabetic cells, the presence of micro- and macroangiopathy or neuropathy. It is interesting to note however, that dysfunctional humoral response reported in a previous study was associated with an unexplained increased levels of immunoglobulin A (IgA) in diabetic subjects that do not have any history of infection.[8] It is thus likely that these subjects may have subclinical infections especially those affecting urinary and intestinal tract or that the elevated IgA levels are secondary to metabolic disturbance of diabetes. Humoral immunity is a complex reaction that involves the interdependent of complements, cytokines and immunoglobulins. Whilst alterations of low complement factor 4, decreased cytokine responses after stimulation have been described in diabetic patients [9], the pattern of expression of the immunoglobulins involved in humoral immune response have not been extensively studied. It is plausible that dysfunctional humoral antibodies that occurs in DM leads to susceptibility to infections as a result of poor glycaemic control or a reaction that occurs when the infection has already set in. The objective of this study therefore was to evaluate the pattern of humoral immune response in Nigerians with Diabetic mellitus and its association with glycaemic control indices.

MATERIAL & METHODOLOGY

Study design: This was a analytical cross sectional study carried over a period of 4 months from May 2015 to September 2015.

Study population: The study population consisted of subjects with DM who were receiving care at the Diabetes Unit of Randle General Hospital, Lagos, and an urban hospital in Nigeria.

Ethical approval: Informed consent was obtained from the study subjects and Ethical approval was given by the Ethics Committee of the hospital.

Inclusion criteria: The study population included150 subjects with type 2 DM aged between 38 and 80 years. A total number of 75 sex and aged matched individuals served as healthy controls.

Exclusion criteria: It included the following; those requiring haemodialysis, people ill enough to warrant hospitalization, pregnant women, persons who met inclusion criteria but did not give consent for the study.

Methodology: Interview administered questionnaire were used to obtain medical history and to record clinical indices. Blood pressure (BP in mm/Hg) of all the subjects was measured on the left arm using Accuson mercury sphygmanometer. An appropriate sized cuff was placed about 2.5 cm above the antecubital fossa with participants sitting, after resting for at least ten minutes. 6.0ml of venous blood samples were collected from each subject in a sitting position after an overnight fast (10 – 24 hours). Plasma samples were collected after centrifugation and analyzed.

Laboratory analyses: The DM free status of the controls was ascertained by having them subjected to glycosylated haemoglobin and fasting plasma glucose tests. They were deemed as not having DM if their fasting plasma glucose and glycosylated haemoglobin were less than 100mg % and 5.7% [10] respectively. Short term, medium term and long term glycaemic control were assessed using fasting glucose, fructosamine and glycosylated haemoglobin respectively. Short - term good glycaemic control was defined as fasting plasma glucose levels ≤ 110mg%. Medium - term good glycaemic control was defined as fructosamine levels ≤ 287.5µmol/l [13]. Long – term good glycaemic control was defined as glycosylated haemoglobin levels ≤ 7% [11]. The controls and the subjects all had biochemical tests done and these included plasma immunoglobulins A, G, and M. Glycosylated haemoglobin (HbA1c) was estimated using chromatographic – spectrophotometric ion exchange method using Biosystems kit, Spain. The name and model of the spectrophotometer used was SSRFI and BSA 3000. Fructosamine was estimated spectrophotometrically, using a kit adopted by Fortress, UK. Fasting plasma glucose was estimated using glucose oxidase method. The immunoglobulins were measured by enzyme linked immunosorbent assay (Elisa) technique using commercial test kits of WKEA (China) for IgG and IgA and AccuDiag (USA) for IgM respectively. The absorbances were read with a micro plate reader (stat fax, USA, model no 2100).

STATISTICAL ANALYSIS

The statistical package used for the analysis was SPSS version 15. Quantitative variables were compared with independent student t test. The comparison within and among groups were done using one way analysis of variance (ANOVA). Correlations were explored with Pearson' correlation coefficient. Standard multiple regression analysis was used to predict outcomes. Quantitative data are expressed as mean ± standard deviation and mean ± standard error of mean. Probability values of less than 0.05 were considered to be statistically significant.

RESULTS

The mean age, standard deviation (SD) and age range of the study subjects were 59.9 (10.2) years and 38 – 80 years

respectively. The number and proportion of the males to females in this study is 36 (23.8%): 114 (76.2%). The proportion of females differed significantly from the males (p = 0.000). A total number of 95 people had hypertension and this made up 63% of the DM subjects. A higher proportion of females 59% than males 41% had hypertension and this difference was statistically significant, p = 0.002. The presence of other co – morbidity and complications in addition to DM subjects with hypertension was noticed in 109 (72.7%) of the study subjects; these included those with musculoskeletal syndrome 2 (1.33), neuropathy 6 (4%), retinopathy 4 (2.67%) and cardiovascular accident 2 (1.33%). The mean levels of IgM were significantly lower in DM subjects compared with controls; 0.82 ± 0.11µg/ml Vs 2.39 µg/ml, p = 0.000. This is shown in table 1. Table 2 shows comparable differences in the humoral immunoglobulins of newly diagnosed DM (less than 5 years) and those with longer duration. Table 3 shows no association between immunoglobulins with duration of illness. An inverse relationship was observed between immunoglobulin G with fructosamine (r = - 0.356, p = 0.030) and glycosylated haemoglobin (r = - 0.352, p = 0.026) (see table 4). All studied humoral antibodies had significantly reduced IgM levels in DM subjects without any form of co – morbidity, when compared with DM + co- morbidity as well as apparently healthy control subjects. These results are shown in table 5. Immunoglobulin M (IgM) showed an inverse association with both systolic and diastolic blood pressures (see table 6).

Table 1. Levels (mean± SEM) of immunoglobulins A, G and M in DM and control subjects

Immuno globulins	DM n = 150	Controls n = 75	t values	pvalues
IgA (µg/ml)	4.99± 0.44	6.25± 0.74	-1.452	0.150
IgG (µg/ml)	11.52± 0.27	10.84± 0.45	1.257	0.212
IgM(µg/ml)	0.82± 0.11	2.39± 0.41	-5.255	0.000*

*Significant

Table 2. Levels (mean ± SEM) of immunoglobulins A, G, and M in DM of less than 5 Years and greater than 5 years duration

Immunoglo bulins	DM<5years n = 85	DM >5 years n = 65	T values	p value
IgA	4.76 ± 0.51	5.45 ± 0.83	-0.750	0.456
IgG	11.33± 0.36	11.9 ± 0.39	-0.985	0.329
IgM	0.88 ± 0.15	0.69 ± 0.12	0.829	0.410

Table 3. Association of Immunoglobulins A, G, and M in DM with Duration of illness using Pearson correlation coefficient

Immunoglobulins	Correlation coefficient r	p values
IgA	0.086	0.514
IgG	0.179	0.161
IgM	-0.186	0.149

Table 4. Correlation of immunoglobulins A, G and M in DM with Glycaemic control indices

Immuno globulins	Fasting Plasma Glucose r (p)	Fructosamine r (p)	Glycosylated Haemoglobin r (p)
IgA	-0.035 (`0.789)	0.086(0.540)	0.094 (0.576)
IgG	-0.101(0.431)	-0.356(0.030)*	-0.352(0.02)*
IgM	0.000 (1.000)	0.020(0.721)	0.014 (0.656)

*Significant

Table 5. Analysis of variance showing within group and between group comparison of the levels (mean ± SEM) of immunoglobulins A, G, and M in DM, DM + co - morbidity and control subjects.

Immuno globulins	DM n = 41	DM + co morbidity n = 109	Controls n = 75	F values	p values
IgA	5.17± 0.6	4.91 ± 0.6	6.09± 0.9	0.691	0.504
IgG	11.8± 0.3	11.36± 0.4	10.9± 0.4	0.910	0.407
IgM	0.68± 0.1	0.88± 0.2	2.70± 0.5	18.025	0.000*

*significant

Table 6. Association of immunoglobulins A, G, and M levels in DM subjects with systolic and diastolic blood pressures using Pearson's correlation coefficient

Immunoglobulins	Systolic Blood Pressure r (p)	Diastolic Blood Pressure r (p)
IgA	-0.17 (0.894)	0.115 (0.379)
IgG	0.072 (0.577)	-0.107 (0.404)
IgM	-0.269 (0.034)*	-0.257 (0.044)*

*Significant

DISCUSSION

Any immune system faced with a potential threat, such as hyperglycaemia tries to respond. Some of these responses may have other devastating effect and could eventually lead to further damage. In this study, we assessed the humoral immune response of Nigerians with type 2 DM, where immunoglobulins A, G and M levels in their peripheral blood were measured and compared with those of healthy controls. Our results showed a significantly reduced plasma level of immunoglobulin M (IgM) in diabetic subjects when compared with healthy controls whilst no significant differences in the levels of immunoglobulins A, and G was observed between the two groups. The results obtained from this study somewhat agrees with other reports by Ardawi et al and Saleh in the pattern of immunoglobulin G expressed [12][13]. In the study by Saleh, the levels of all the humoral response antibodies (IgA, IgG and IgM) were decreased. Whilst imuunoglobulin G accounts for 70 – 75 % of the total serum immunoglobulin pool, IgM accounts for about 10% and IgA is the predominant immunoglobulin in sero – mucous secretions. It is interesting to note that evidence' regarding the pattern of immunoglobulins A, G and M of DM in literature has been inconsistent. This could be attributed to the fact that serum immunoglobulin levels are dependent on a variety of conditions such as genetics, chronic disease and environmental factors. These also include ethnic back ground, age, and sex, history of allergies or recurrent infections, and geographic factors [14]. It is instructive to note

that immunoglobulin M is the first antibody formed in the primary immune response and is largely confined to the intravascular pool. This may aptly explain why it is the first antibody to be affected by the glucotoxic microenvironment created in diabetes. The reduced immunoglobulin level is a consequence of a decrease in the percentage of activated (CD38+) B-cells found in diabetic patients which may contribute to the reduced humoral immune response observed in DM [15]. The mechanism by which antibodies further modulates the immune response in DM are not completely defined. It is postulated that IgM antibody together with antigens specifically enhance the immune response of that antigen, whereas IgG antibody suppresses the response. [16]

In our report, we found no association between immunoglobulins A, G and M with duration of DM while poor glycaemic control (fructosamine and glycosylated haemoglobin) was inversely associated with only IgG. An Immunoglobulin G is the classical gamma globulin, which is the major circulating antibody. This immunoglobulin appears about 24-48 hours after antigenic stimulation and continues antigen antibody interaction already begun by Immunoglobulim M. This may possibly explain why it is majorly affected by poor glycaemic control compared with other immunoglobulins. It is instructive to note however that it is actually the IgM – containing immune complexes that are taken up by the fragment crystallisable (Fc) or Complement 3 receptor on antigen presenting cells and are processed more efficiently when compared with IgG. Additionally, other studies have shown with respect to the biological activity of Immunoglobulin G from diabetics that certain functional properties of the Fc region of IgG are impaired; i. e. a decrease in binding of proteins and fixation of complement to the Fc fragment. These probably contribute to the increased susceptibility to infections, known to occur in poorly controlled diabetics. The reasons for the changes in the functional properties of the immunoglobulins are unknown. It is plausible that oxidation of amino acids by free radical mechanism is responsible for the damage of the complement binding site, leading to an alteration of biological activity. The glycation of nuclear acids may be the cause of DNA mutations and could alter its capacity for replication and transcription. Interaction with proteins and fixation of complement depend on the integrity of the region of the heavy chains.

We have also shown in this study that over half of our patients with DM also had hypertension (63%). This is more common than other co – morbidities and complications observed. Hypertension, a cardiovascular risk factor and metabolic syndrome defining criterion is a commonly documented co – morbidity of DM in Nigerians. It is instructive to note that the presence of hypertension, with regards to systolic and diastolic blood pressure associated inversely with IgM, while DM subjects with other complications had an increased levels of IgM when compared with those without any complications.

CONCLUSION

Plasma levels of IgM immunoglobulins are lower in subjects with DM than in people without DM and while IgG and IgA are comparable in DM and healthy controls. Plasma IgG and IgM levels are significantly and inversely associated with glycaemic control indices and blood pressures respectively.

ACKNOWLEDGMENTS

We wish to acknowledge the Nurses and members of staffs of Diabetes Unit of Randle General Hospital, Lagos. Nigeria.

Competing interests: The authors declare that there were no competing interests.

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