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Altered immunoglobulins (A and G) in Ghanaian patients with type 2 diabetes

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Abstract

Objectives: Elevated immunoglobulin levels have been strongly linked to the development and progression of inflammatory disorders such as type 2 diabetes and obesity. This study aimed to evaluate circulating immunoglobulin levels and to identify other metabolic factors that influence humoral immune response among Ghanaian subjects with type 2 diabetes.

Methods: A comparative cross-sectional study conducted at the National Diabetes Management and Research Center, Accra. Eighty persons with type 2 diabetes were age-matched with 78 controls. Immunoglobulin A, immunoglobulin G and immunoglobulin M; interleukin 6; fasting blood glucose; glycated hemoglobin; and lipid parameter concentrations were measured. Blood pressure, anthropometry and body composition indices were also assessed.

Results: Median immunoglobulin A and immunoglobulin G (g/L) levels were higher in the case group compared with controls (0.89 vs 0.74, $p = 0.043$; 7.58 vs 7.29, $p < 0.001$). Immunoglobulin G, immunoglobulin A and interleukin 6 levels in the case cohort, respectively, associated weakly with fasting blood glucose ($r = 0.252$, $p = 0.001$; $r = 0.170$, $p = 0.031$; $r = 0.296$, $p = 0.001$). There were positive correlations within the control group for immunoglobulin A versus interleukin 6 ($r = 0.366$, $p = 0.001$) and within the case group for glycated hemoglobin versus interleukin 6 ($r = 0.190$, $p = 0.020$).

Conclusion: Our data suggest that humoral immune response is altered in subjects with type 2 diabetes and that serum immunoglobulin levels could serve as useful biomarkers in the investigation and management of diabetes mellitus.

Keywords

Immunoglobulin, interleukin, type 2 diabetes

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Introduction

Serum immunoglobulin levels play a significant role in the body's defense against pathogens. There are five classes of immunoglobulins: immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin D (IgD) and immunoglobulin E (IgE). Immunoglobulin concentrations tend to increase with age[1] or exposure to pathogens (antigens).[2] Studies have also reported changes in serum immunoglobulin levels among subjects with type 2 diabetes.[1,3–4] Pro-inflammatory cytokine, interleukin 6 (IL-6), plays an important role in the mediation of inflammatory response[5–8] and is also involved in the development and acceleration of microvascular complications in patients with diabetes mellitus.[9] The extent to which these circulating immunoglobulins influence metabolic dysfunction is

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not fully known particularly with regard to ethnicity. The purpose of this study was primarily to investigate possible immunological alterations associated with persons with type 2 diabetes and to identify which other factors influence humoral immune response in Ghanaian subjects with and/or without type 2 diabetes.

Methods

Study site, design, participants and exclusion criteria

This was a comparative cross-sectional study. Participants included 80 persons with type 2 diabetes, attending the National Diabetes Management and Research Center (NDMRC), Korle-Bu, Accra, and 78 age- and gender-matched staff/workers of the Korle-Bu Teaching Hospital, Accra, Ghana, without diabetes mellitus. An oral glucose tolerance test (OGTT), regarded as diagnostic screening for type 2 diabetes, was performed on all volunteers. Anthropometric measurements such as height and weight were taken and body mass index (BMI) was calculated. Blood pressure was taken using a mercury sphygmomanometer and stethoscope after participants had rested for 15 min. Type 2 diabetes was confirmed at the center (NDMRC) based on results of fasting blood glucose (FBG) $\leq$ 6.9 mmol/L and a 2-hr OGTT $>$ 11.1 mmol/L on two separate occasions. Type 2 diabetic persons were either being lifestyle managed or were on oral hypoglycemic drugs. A pre-tested structured questionnaire was administered to assess the socio-economic status, medical history, medications and level of physical activity of subjects. The study was approved (Protocol Identification Number: MS-Et/M.2–P4.9/2013-2014) by the Institutional Ethics and Protocol Review Committee of the School of Medicine and Dentistry, College of Health Sciences, University of Ghana. Detailed explanations on purpose of the study, risk and benefits were made known to participants. Written informed consent was obtained from all participants. Subjects who have been smoking and drinking alcohol continuously for 6 months were excluded from the study. Subjects who were immunosuppressed such as those with immunoglobulin deficiency syndrome, HIV and hepatitis B were also excluded from the study. Participants who tested positive for the urine nitrite test or had bacterial and parasitic infections were also excluded from the study. The above have been proven to affect immunoglobulin levels in subjects.[10] For minimum sample size determination, we established that 130 persons (65 persons for each study group) was adequate for this study, using a 6.3% prevalence rate for diabetes mellitus in Ghana,[11] at 95% confidence interval and assuming a marginal error of 6%.

Laboratory procedure

Venous blood (9 mL) was obtained from the subjects between 7 and 9 a.m. each day, after an overnight fast, according to Helsinki protocol declaration (2008). Two milliliters of whole blood was transferred into sodium fluoride containing tube and the plasma separated for the estimation of glucose. Three milliliters of whole blood was further transferred into ethylenediaminetetraacetic acid (EDTA) containing tubes for the estimation of glycated hemoglobin (HbA1c). The remaining 4 mL of whole blood was further processed, and resulting sera were then aliquoted in 1 mL portions into sterile Eppendorf Tubes and stored at −20°C until analyzed.

Early morning spot urine samples from study subjects were collected into sterile plastic universal urine containers for urinalysis. FBG, total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterols were analyzed using the VITROS system chemistry auto-analyzer (version 250) (Ortho Clinical Diagnostics [version 5, 1 FS], Rochester, New Jersey, USA). HbA1c determination was based on a latex agglutination inhibition assay (Randox Laboratories Ltd, Kearneysville, WV, USA). The human IgA, IgG and IgM enzyme-linked immunosorbent assay (ELISA) Kits (USCN Life Science Inc., Wuhan, China) were used for in-vitro quantitative measurement. IL-6 was analyzed using a kit purchased from Kamiya Biomedical Company, Tukwila, WA, USA. Specific secondary antibodies were used to eliminate unspecific binding to substrates. Calibration curves were used to determine analyte concentrations from the strength of signal produced by each immunoassay.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS Software, San Diego, CA, USA) was used for analysis with level of statistical significance set at $p < 0.05$ for all tests. Values are expressed as mean plus or minus standard deviations and also as medians with ranges where appropriate The Wilcoxon ranked sum testing was used to assess immunoglobulin and IL-6 levels in the study population. The unpaired Student's t-test was used to evaluate differences between two means. Pearson product moment correlation coefficient (r) was used to find the association between two continuous variables.

Results

The clinical and biochemical parameters of the study population are shown in Table 1. Eighty persons with type 2 diabetes were age-matched with 78 non-diabetic controls. BMI, waist circumference (WC), HbA1c and FBG between the two study groups were statistically significant ($p < 0.05$). Comparison between the two study groups did not reveal any difference ($p > 0.05$) for systolic blood pressure (SBP), diastolic blood pressure (DBP), TC, TG, HDL and albumin. The median IgA, IgG and IL-6 concentrations were significantly higher ($p < 0.05$) in persons with type 2 diabetes compared with controls. Median values for IgM, between the two study

Table 1. Clinical and biochemical parameters of study population.

Parameters	Type 2 diabetes (N = 80)	Controls (N = 78)	p-value
Age (years)	50.6 ± 7.4	48.1 ± 7.1	0.061
M/F	38/42	38/40	–
BMI (kg/m^2)	29.7 ± 5.8	25.1 ± 4.1	**0.001**
WC (cm)	99.7 ± 15.2	88.5 ± 11.4	**0.001**
SBP (mm Hg)	137.8 ± 17.2	132 ± 19.8	0.080
DBP (mm Hg)	82.7 ± 10.1	78.8 ± 19.2	0.112
FBG (mmol/L)	8.7 ± 3.7	4.3 ± 0.7	**0.001**
HbA1c (%)	7.2 ± 1.3	5.6 ± 0.9	**0.019**
TC (mmol/L)	5.0 ± 1.4	5.2 ± 1.1	0.184
TG (mmol/L)	1.2 ± 0.6	1.2 ± 0.9	0.983
HDL (mmol/L)	1.3 ± 0.5	1.3 ± 0.3	0.467
LDL (mmol/L)	3.1 ± 1.3	3.3 ± 0.9	0.156
T. protein (g/L)	76.2 ± 8.1	79.1 ± 4.8	**0.007**
Albumin (g/L)	44.0 ± 3.9	46.5 ± 4.2	0.188
Period of diabetes (years)	8.6 ± 5.4	–	–
IgA (g/L)	0.89 (0.36−4.81)	0.74 (0.34−3.51)	**0.043**
IgG (g/L)	7.59 (6.43−8.35)	7.29 (4.68−11.65)	**0.001**
IgM (g/L)	0.73 (0.08−4.99)	0.57 (0.01−4.06)	0.263
IL-6 (pg/mL)	1.70 (0.39−14.93)	0.99 (0.01−4.52)	**0.001**

M: male; F: female; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBG: fasting blood glucose; HbA1c: glycated hemoglobin; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; BMI: body mass index; T. protein: total protein; WC: waist circumference; IL: interleukin; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M.
Values are given as mean ± standard deviation and also as medians with minimum–maximum ranges (within parenthesis). Mean difference is significant ($p < 0.05$). Bolded results indicate significant relationships.
$p < 0.05$ is statistically significant.

groups, were not statistically significant (p = 0.270) (Table 1). Associations between several parameters (age, BMI, SBP, FBG, HbA1c, LDL and HDL) with IgA, IgG, IgM and IL-6 have been shown in Table 2. There were negative associations for HbA1c versus IgG (r = −0.220, p = 0.013) and positive correlation for BMI versus IgG (r = 0.161, p = 0.043), FBG versus IgA (r = 0.170, p = 0.031), FBG versus IgG (r = 0.252, p = 0.001), HbA1c versus IL-6 (r = 0.190, p = 0.020), IL-6 versus IgA (r = 0.326, p = 0.002) and IL-6 versus IgM (r = 0.177, p = 0.031), respectively, within the case group. Control group had a negative relationship for HbA1c versus IgG (r = −0.281, p = 0.012) and a positive association for IL-6 versus IgA (r = 0.366, p = 0.001).

Discussion

This study sought to investigate serum levels of immunoglobulins (IgA, IgG and IgM) and IL-6 in Ghanaian persons with type 2 diabetes. Even though the distribution of the three immunoglobulins did not follow normal distribution and was skewed, the ranges for immunoglobulin levels measured for persons without type 2 diabetes in this study were consistent with established ranges[12] for healthy individuals. Results from this study generally did not agree with previous findings.[1,4] Our data showed that concentration of IgG was greater than the other immunoglobulins (IgA and IgM) in both persons with and without type 2 diabetes, confirming primarily the existing information that IgG is the most abundant circulating immunoglobulin in humans.[2] Again, the observed significantly higher IgG concentrations among subjects with type 2 diabetes compared with their control counterparts could be a result of chronic metabolic dysfunction with accompanying low-grade inflammation.[13]

Concentrations of IgA in this study were significantly higher in the case cohort. This observation may reflect the possible accumulation of inflammatory conditions associated with type 2 diabetes.[1] Furthermore, IgA is reported to be a non-specific marker in the development of diabetic complications.[4] IgA levels also associated significantly with serum concentrations of IL-6, a marker of inflammation and a co-factor for immunoglobulin synthesis.[1] Therefore, monitoring IgA may provide early warning of possible presence of complications. Serum IgM levels in this study were not different in both study groups and was consistent with prior studies.[1,3] Serum IL-6 levels were significantly higher in subjects with type 2 diabetes than their non-diabetic counterparts. This finding agreed with earlier observations,[14,15] but contradicted another study.[16] It has been reported that elevated levels of IL-6 independently increases the risk of developing type 2 diabetes by diminishing insulin sensitivity.[6,17] This study, however, was unable to measure the insulin resistant state of participants. IL-6 has also been implicated in the development and acceleration of microvascular complications in

Table 2. Association of several correlates with IgA, IgG, IgM and IL-6.

Variables		Case group (type 2 diabetes (80))				Control group (non-diabetics (78))			
		IgA (g/L)	IgG (g/L)	IgM (g/L)	IL-6 (pg/mL)	IgA (g/L)	IgG (g/L)	IgM (g/L)	IL-6 (pg/mL)
Age	r	**0.159**	0.036	0.047	0.114	0.133	−0.200	−0.042	0.223
	p	**0.047**	0.065	0.558	0.154	0.245	0.079	0.714	0.051
SBP	r	0.018	−0.042	0.023	0.054	0.065	−0.070	0.118	−0.129
	p	0.824	0.600	0.775	0.503	0.572	0.541	0.304	0.911
BMI	r	0.025	**0.161**	0.069	0.077	−0.163	−0.058	0.181	−0.046
	p	0.751	**0.043**	0.388	0.337	0.155	0.614	0.113	0.691
FBG	r	**0.170**	**0.252**	0.050	**0.296**	0.047	0.015	0.061	0.072
	p	**0.031**	**0.001**	0.530	**0.001**	0.683	0.899	0.595	0.530
HbA1c	r	−0.035	**−0.220**	0.089	**0.190**	−0.141	**−0.281**	−0.166	0.050
	p	0.662	**0.013**	0.265	**0.020**	0.219	**0.012**	0.146	0.665
TC	r	0.110	0.070	−0.042	0.032	0.064	−0.013	−0.016	−0.141
	p	0.168	0.389	0.604	0.679	0.578	0.908	0.891	0.219
LDL	r	−0.090	−0.027	−0.108	0.045	−0.034	−0.070	0.054	−0.144
	p	0.260	0.750	0.176	0.541	0.770	0.564	0.643	0.207
HDL	r	−0.053	0.250	0.028	**0.169**	−0.111	0.048	−0.168	−0.043
	p	0.506	0.529	0.723	**0.047**	0.335	0.679	0.142	0.706
IL-6	r	**0.326**	0.054	**0.177**		**0.366**	0.034	0.074	
	p	**0.002**	0.498	**0.031**		**0.001**	0.765	0.519	

SBP: systolic blood pressure; FBG: fasting blood glucose; HbA1c: glycated hemoglobin; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; BMI: body mass index; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; IL-6: interleukin 6.
Pearson's $r < 0.5$ shows weak positive correlation. Bolded results indicate significant relationships.

patients with diabetes mellitus.[9] HbA1c and FBG in this study were higher in persons with type 2 diabetes compared with the control group and positively correlated with IL-6 suggesting an inflammatory crosstalk in glucose regulation.[18,19] SBP and DBP in this study, were not different between the two study groups. According to Amoah et al.,[20] chronic glycemic status tended to be associated with increase in SBP and DBP. Persons with type 2 diabetes in this study were, however, on prescribed medication and such management practice could have been responsible for such observation. BMI and WC in this study were found to be significantly higher in the case group compared to controls. High BMI and WC are markers of adiposity and have the potential for the development and progression of type 2 diabetes.[21–25]

This study had some limitations. Although the authors primarily focused on overweight subjects, a normal weight control group would have been useful in a cross-sectional comparison manner. Another limitation was our inability to measure the insulin resistant state of our subjects. Furthermore, the authors did not have complete information regarding type 2 diabetic complications. Future studies should focus on large population-based longitudinal studies to understand the mechanistic role of immunoglobulins and inflammatory markers in insulin resistance and other metabolic disorders. In conclusion, circulating immunoglobulin levels could serve as useful biomarkers in the investigation and management of diabetes mellitus. Implications of humoral responses in diabetes mellitus merit further investigation.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

The study was approved (Protocol Identification Number: MS-Et/M.2–P4.9/2013-2014) by the Institutional Ethics and Protocol Review Committee of the School of Medicine and Dentistry, College of Health Sciences, University of Ghana.

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Informed consent

Written informed consent was obtained from all subjects before the study.

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