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Original Article

Acute phase reactant dynamics and incidence of microvascular dysfunctions in type 2 diabetes mellitus

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Abstract

BACKGROUND: Acute Phase Reactants (APRs) have a wide range of activities that contribute to host defense. The aim of this report was to evaluate the dynamics and magnitude of these proteins in various microvascular complications in diabetes mellitus (DM). We also sought to assess the predictive values of APRs and other clinical variables for microvascular complications in DM.

METHODS: This was a case control study carried out in 200 Nigerian subjects with type 2 DM and 100 sex and age matched healthy controls. The studied APRs included C-reactive protein, beta 2 microglobulin, fibrinogen and lipoprotein (a).

RESULTS: The mean values of the APRs were significantly higher in type 2 DM compared with the controls and were observed in higher concentrations in those with microvascular complications, except beta 2 microglobulin. Presence of microvascular complications was observed in those with dilated fundus examination (retinopathy), symptom score of 3.0 (neuropathy), urea and creatinine levels above 50mg% and 1.5mg%, respectively, with significant proteinuria (nephropathy). Significant increase in mean ± SEM values of lipoprotein (a) was observed in diabetic retinopathy in comparison with those without complications (25.76 ± 1.13 mg/dl vs. 22.37 ± 0.73 mg/dl, p = 0.005). Elevated C-reactive protein was observed in diabetic neuropathy in comparison with those without complications (11.43 ± 2.33 u/ml vs. 8.30 ± 1.15 u/ml, p = 0.048). Increased beta 2 microglobulin levels were observed in patients with diabetic foot ulcers in comparison with those without complications (3.04 ± 0.51 mg/dl vs. 2.54 ± 0.14 mg/dl, p = 0.049). Circulating levels of Lipoprotein (a) predicted retinopathy in DM with both good and poor long-term glycemic control while duration of DM predicted the occurrence of foot ulcers..

CONCLUSIONS: Increased level of APRs was associated with a number of microvascular complications and may play a role in the pathogenesis.

KEYWORDS: Acute Phase Reactants, Type 2 Diabetes Mellitus, Microvascular Complications.

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Microvascular dysfunctions in diabetes are due to abnormalities in small blood vessels, and particularly affect the retina (diabetic retinopathy) and the kidney (nephropathy).[1] It is equally important to consider the acute phase response, which is a dynamic homeostatic process that involves all the major systems of the body in addition to the immune, cardiovascular and central nervous system. Normally, the acute phase response lasts only a few days. However, in cases of chronic or recurring inflammation, an aberrant continuation of some aspects of the acute phase response may contribute to the underlying tissue damage that accompanies the disease, and also leads to further complica-

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tions. Acute phase reactants have a wide range of activities that contribute to host defense; they can directly neutralize inflammatory agents, help to minimize the extent of local tissue damage, as well as participate in tissue repair and regeneration. Changes in these acute phase reactants are not specific and the non specific nature of the response means that individual proteins are rarely helpful as an aid in diagnosis; though some like C reactive protein have been strongly associated with inflammatory response.[2]

Levels of acute phase reactants have been found to be elevated in adult diabetes,[3,4] but normal in childhood diabetes,[5] suggesting that the increase could be related to the occurrence of microvascular dysfunctions rather than the diabetes per se. Studies have shown increased levels of acute phase markers in nephropathy and also in patients with microalbuminuria.[6,7] Increased C-reactive protein (CRP) was observed in Diabetes mellitus.[8] Fibrinogen was reported to be associated with both cardiovascular risk and nephropathy in type 1 and 2 diabetes.[9,10] Hyperfibrinogenemia was reported in Nigerian diabetics, although there was no sufficient evidence to suggest its role in organ complications. Diabetic patients were also reported to have a higher lipoprotein (a) [Lp(a)] than non diabetic persons.[11-13] However, data on the status of Lp (a) in Nigerian diabetics is not substantive. Serum beta 2 microglobulin (β2MGB) levels in DM have not been well documented, though the pathophysiology of this acute phase reactant with regards to organ complications is still obscure. An intriguing report had earlier suggested a clinical importance in decreasing this protein.[14] The implication of the changes in the pattern of acute phase reactants in DM with microvascular complications is poorly understood. The aim of this study was to evaluate the dynamics and magnitude of these acute phase reactants in various microvascular dysfunctions of type 2 DM. We also sought to assess the predictive values of APRs and other clinical variables for microvascular complications.

Methods

This was a case control study carried out at the Diabetes Centre of the Lagos State University Teaching Hospital, Ikeja, Lagos State, Nigeria. A total of 200 subjects with type 2 DM and 100 healthy age and sex matched individuals served as controls. The control subjects were volunteer staffs of the hospital and were screened for DM by having them subjected to fasting plasma glucose and glycosylated hemoglobin tests. Venous samples were collected from the antecubital fossa of all the subjects after an overnight fasting (10-14 hours) in a sitting position. Duration of the illness was grouped into early stage (1-5 years) and late stage (greater than 5 years). The DM subjects were being managed with oral hypoglycemic agents and diet at the time of study. The subjects were further subdivided on the basis of presence or absence of microvascular complications. Dip stick urinalysis was carried out on all subjects as well as controls to rule out infective proteinuria when their urine tests were positive for nitrite and leucocytes. Elevated serum urea and creatinine in addition to proteinuria and clinical presentation was used to categorize DM with renal failure due to diabetic nephropathy. Neuropathy was established using the diabetic neuropathy symptom score, using a cut-off point of 3.0.[15] This was based on the ability to respond to three of four questions, which includes queries about numbness to the feet or legs, pricking sensation of the feet or legs, burning or aching of the feet or legs, gait problems and pain. Retinopathy was observed in those with dilated fundus examination. Ethical approval was received from the Ethics and Research Committee of Lagos State University Teaching Hospital. Consent of the patients was gotten through the aid of a well structured questionnaire before the commencement of the study.

Fasting blood samples were collected into tubes containing fluoride oxalate, lithium heparin, EDTA and citrate anticoagulant. Other exclusion criteria for both subjects and controls included those on immunosuppressive drugs, those with systemic diseases, those on steroids,

those with malignancies, heart failure patients, pregnant women, and those who were undergone surgery recently. Excluded subjects were also those with malaria parasite and those positive for rheumatoid arthritis.

Biochemical analysis: C-reactive protein, beta 2 microglobulin and Lp(a) was measured turbimetrically, while fibrinogen estimation was done using clot weight method of ingram.[16] Glucose was determined by the glucose oxidase method.[17] The elevated Lp(a) was defined as serum levels above 30 mg/dl,[18] high CRP as levels above 3 mg%[19] And hyperfibrinogenemia as fibrinogen levels of 0.35 mg/l.[20] Elevated β2GMB levels was defined as serum levels above 3 mg%.[14]

Statistical Analysis

Data were analyzed using SPSS software version 15 and Epi Info. Independent student's t-test and chi-square were used to compare groups. Within groups and between groups comparison was done using analysis of variance (ANOVA). Quantitative data are expressed as mean ± standard error of mean (SEM). Probability values of less than 0.05 were considered to be statistically significant ($p < 0.05$). Logistic regression analysis was used to predict outcomes.

Results

The mean duration of DM in the study subjects was 6 ± 0.59 years ranging from 0.1 to 10 years. The mean age of the DM subjects (57.7 ± 0.76 years) and controls (59.6 ± 0.89 years) was comparable ($p = 0.8$) and the female-male ratio in both was 2:1. The DM free controls had fasting plasma glucose and glycosylated haemoglobin less than 100 mg% and 5.7% respectively.[21] The level of acute phase reactants in diabetic subjects were significantly higher than those in control group (Table 1).

The prevalence of microvascular complications in the 200 diabetic participants were as follows: retinopathy 20% (n = 40), neuropathy 31% (n = 62), nephropathy with renal failure 1% (n = 2), and foot ulcers 7.5% (n = 15). Within group and between group analysis using one way ANOVA showed that increased levels of the acute phase reactants were observed in DM subjects with microvascular complications in contrast to those without microvascular complications as well as the controls, except for beta 2 microglobulin (Table 2). The acute phase reactant concentrations were observed to be lower in DM with shorter duration of illness (less than five years) when compared with those of longer duration, except CRP (10.04 ± 1.22 Vs 7.94 ± 1.50, $p = 0.285$). Using relevant cut-off points where applicable; retinopathy was detected in 78% and 53% of subjects with elevated Lp(a) and CRP, respectively; neuropathy was seen in 68% of subjects with elevated CRP and presence of foot ulcers was observed in 77% of participants with elevated β2 MGB (Table 3).

Table 1. Comparison of acute phase reactants, serum urea and creatinine levels of type 2 diabetic patients with control subjects.

Acute phase reactants	DM Patients mean± SEM n = 200	Controls mean± SEM n= 100	t	P-value
C-reactive protein (u/ml)	9.27 ± 0.95	3.37 ± 0.20	4.400	<0.001
Beta 2 microglobulin (mg/ml)	2.58 ± 0.09	1.63 ± 0.05	6.904	<0.001
Fibrinogen (g/dl)	1.93 ± 0.04	1.23 ± 0.05	11.054	<0.001
Lipoprotein(a) (mg/dl)	23.05 ± 0.48	18.81 ± 0.59	5.280	0.001
Urea (mg%)	44 ± 0.97	35 ± 1.00	0.980	0.244
Creatinine (mg%)	1.30 ± 0.02	0.90 ± 0.04	0.7450	0.200

Table 2. Comparison of diabetic patients without microvascular complications, diabetic patients with microvascular complications and control subjects.

Acute phase reactants	Controls n = 100	DM with microvascular complications n = 81	DM without microvascular complications n = 119	f	P-values
C-reactive protein (u/ml)	3.37 ± 0.20	8.88 ± 1.22	9.74 ± 1.47	9.819	<0.001
Beta 2 microglobulin (mg/ml)	1.63 ± 0.05	2.51 ± 0.14	2.67 ± 0.13	0.990	0.373
Fibrinogen (g/dl)	1.23 ± 0.05	1.87 ± 0.04	2.05 ± 0.05	60.062	<0.001
Lipoprotein(a) (mg/dl)	18.84 ± 0.60	22.63 ± 0.70	23.62 ± 0.68	14.50	<0.001

The proportion of DM patients with good long term glycemic control as reflected in their glycosylated hemoglobin was 68% while 32% had poorly controlled glycemia. Logistic regression analysis of the APRs with various microvascular complications showed that Lp(a) only predicted retinopathy in those with good glycemic control (odds ratio = 1.065, 1.005 to 1.128 95% confidence interval, p = 0.03) as well as in those with poor glycemic control (odds ratio = 1.073, 1.015 to 1.134 95% confidence interval, p = 0.013). The duration of DM also predicted development of foot ulcers (Tables 5 and 6).

Discussion

In this study, the APRs of type 2 DM were all significantly higher than the control group and the concentrations were higher in complicated DM, except for β2MGB. Increased level of CRP observed in this study was in agreement with previous report.[22] This is indicative of inflammation and tissue damage. Hyperfibrinogenemia observed in Nigerian Diabetics is in consonance with some previous studies.[23,24] The adverse effect of this may lead to an imbalance in homeostatic mechanism and may further degenerate to a hypercoagulable state. In addition, elevated Lp(a) levels could be a pointer to cardiovascular predisposition, which conveys the impression that these patients are more prone to atherogenesis than the control cases. This molecule carries a protein that may deter the body's ability to dissolve blood clots and it is under investigation as either a marker or cause of heart disease. Lp(a) appears to be poorly cleared from the plasma and is strongly and independently associated with atherosclerosis.[25] The homology of Lp(a) with plasminogen has led to the hypothesis that it somehow interferes with plasminogen activation and thus impairs fibrinolysis.[26] Until recently, much interest has been focused on Lp(a), which is a complex lipoprotein consisting of lipids, carbohydrates and two larger apoproteins, (b) and (a).

CRP demonstrated a positive relationship with fasting blood glucose. This in comparison with other APRs responded earlier to the glucotoxic microenvironment created in DM. The duration of DM did not affect the pattern of expression of the APRs, only CRP was observed to be insignificantly elevated; this may be as a result of being an early marker of inflammation. This was in contrast to other APRs which could thus be regarded as the late inflammatory markers.

Table 3. Prevalence of elevated acute phase reactants in various microvascular complications.

Microvascular Complications	CRP	β2MGB	Lp(a)	Fibrinogen
Retinopathy	53%	28%	79%	28%
Neuropathy	68%	31%	23%	26%
Foot Ulcers	53%	67%	20%	38%
Nephropathy	34%	39%	43%	44%

LP(a), Lipoprotein (a); **β2MGB**, Beta 2 microglobulin; CRP, C-Reactive protein

Table 4. Logistic regression analysis of acute phase reactants in type 2 DM with good long-term glycemic control (HBA1c < 7%) and various microvascular complications.

Acute phase reactants	Retinopathy OR (95% CI)	P-value	Neuropathy OR (95% CI)	P-value	Foot ulcers OR (95% CI)	P-value	Nephropathy OR (95% CI)	P-value
CRP	1.018 (0.989-1.047)	0.220	1.005 (0.979-1.03)	0.689	1.014 (0.974-1.056)	0.486	1.003 (0.927-1.08)	0.927
B2MGB	0.770 (0.541-1.096)	0.147	1.106 (0.836-1.46)	0.482	1.601 (0.846-3.023)	0.148	1.259 (0.558-2.84)	0.579
Lp(a)	1.065 (1.005-1.128)	0.03*	0.978 (0.927-1.03)	0.405	0.981 (0.872-1.10)	0.753	0.972 (0.817-1.15)	0.l752
Fibrinogen	0.769 (0.362-1.631)	0.494	1.272 (0.637 -2.54)	0.496	0.388 (0.873 -1.10)	0.220	2.17 (0.259– 18.0)	0.476
Sex	1.519 (0.318-7.246)	0.600	0.444 (0.113-1.74)	0.244	2.673 (0.499-14.29)	0.251	0.000 (0.000 -0.00)	0.989
Age	1.160 (0.285-5.217)	0.846	1.334 (0.413-4.30)	0.630	0.803 (0.139-4.619)	0.806	0.000 (0.000 -0.00)	0.988
Duration	1.386 (0.149-12.92)	0.775	0.924 (0.191-4.48)	0.922	1.014 (0.102-10.11)	0.991	0.000 (0.000 -0.00)	0.983

LP(a), Lipoprotein (a); **β2MGB**, Beta 2 microglobulin; CRP, C-Reactive protein; OR, Odds Ratio; CI, Confidence Interval

Of importance to note is that these APRs may be strongly linked to the pathogenesis of organ complications in DM. Lp(a) levels was observed to be significantly raised in diabetic patients with retinopathy. This observation agrees with previous study by Khare et al.[27] Increased advanced glycation end products may account for the increased risk of diabetic complications in patients with higher Lp(a) values.[28] A recent study showed that the levels of APRs correlated with the number of microvascular complications; however, their study did not look at retinopathy but at microvascular complications in general.[29] In this research, a detailed study of APRs was made and thus was able to also observe increased CRP in diabetic neuropathy with a percentage of 53%, increased β2MGB in foot ulcers (67%) and elevated Lp(a) in retinopathy (72%). β2MGB has been found to be associated with falling CD4 count in HIV seropositive individuals, suggesting a close association with suppressed immunity.[27] Increased activation or destruction of lymphocytes raises β2MGB levels.

Table 5. Logistic regression analysis of acute phase reactants in type 2 DM Patients with poor long-term glycemic control (HBA1c > 7%) and various microvascular complications.

Acute phase reactants	Retinopathy OR (95% CI)	P-value	Neuropathy OR (95% CI)	P-value	Foot ulcers OR (95% CI)	P-value	Nephropathy OR (95% CI)	P-value
CRP	1.004 (0.942-1.069)	0.909	1.028 (0.982-1.078)	0.239	0.955 (0.839 -1.088)	0.251	0.994 (0.000-17.6)	0.999
B2MGB	1.177 (0.679 -2.041)	0.561	0.936 (0.615-1.423)	0.756	1.578 (0.724 -3.440)	0.676	0.217 (0.000-0.00)	0.998
Lp (a)	1.073 (1.015 -1.134)	0.01*	1.009 (0.984-1.035)	0.497	0.969 (0.839-1.120)	0.676	0.832 (0.000 -3.37)	0.998
Fibrinogen	0.941 (0.337- 2.626)	0.908	1.332 (0.578-3.068)	0.501	1.143 (0.293-4.461)	0.847	0.109 (0.000-0.000)	0.998
Sex	1.791 (0.725-4.261)	0.188	1.982 (0.876-4.48)	0.101	0.803 (0.159-4.044)	0.987	0.000 (0.000-0.000)	0.790
Age	0.655 (0.196-2.179)	0.490	0.802 (0.271-2.37)	0.690	2.284 (0.379-13.76)	0.367	4.112 (0.244-69)	0.326
Duration	2.003 (0.556-7.221)	0.288	0.883 (0.304-2.56)	0.819	0.125 (0.023-0.683)	0.016	580E+0.03 (0.000-0.00)	0.985

LP(a), Lipoprotein (a); **β2MGB**, Beta 2 microglobulin; CRP, C-Reactive protein; OR, Odds Ratio; CI, Confidence Interval

Since β2MGB is essential for the expression of major histocompatibility complex (MHC) antigens, its stimulation enhances the recognition of bacteria in association with MHC. This could have also being stimulated in response to bacteria which are causative agents in most foot ulcers, and may thus be very vital in combating infectious agents. Our observation agreed with a previous study which observed circulating levels of APRs to be associated with amputation risk of diabetic foot ulcers and thus could aid clinicians in the management of diabetic foot syndromes.

Logistic regression analysis was carried out to evaluate the possibility of using acute phase reactants and other clinical variables in predicting the future development of microvascular complications in DM patients yet to have microvascular complications. This was assessed in DM patients with good long-term glycemic control (HBA1c < 7%) as well as in those with poor long-term glycemic control (HBA1c > 7%). The independent variables entered into the model included the acute phase reactants, sex, age and duration of illness, while the various microvascular complications served as the dependent variables. In this study, Lp(a) only predicted retinopathy in DM with both good and poor long-term glycemic control. It should be emphasized that in addition to Lp(a) being an independent indicator of the risk of vascular disease development,[30] increased levels of this protein proved to be a strong predictor of retinopathy in DM patients as observed in this study. The level of Lp(a) is genetically determined, and when elevated, cannot be lowered by alterations in food intake or by most of the cholesterol lowering agents.[31] This entity could also contribute to the genetic predisposition of some DM patients to complications regardless of good glycemic control.

It was noted that age had no relationship with the occurrence of microvascular complications; rather the duration of the illness that predicted the future development of foot ulcers. This may be secondary to sensory neuropathic changes. Every part of the peripheral nervous system is vulnerable to diabetic involvement. Minor reversible damage can be identified early while late complications as manifested by numbness (anesthesia) and abnormal sensation (paresthesia) and leading to foot ulceration is very common.[32]

Conclusion

Increased levels of APRs correlate with a number of microvascular complications and may play a role in the pathogenesis.

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Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

AA designed the study, participated in the data collation, statistical analysis, funding and writing the draft of the manuscript. OAO participated in the study, subjects selection, funding and data collation. NEA and AOA participated in the study and data collation.

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