Int J Biol Med Res. 2011; 2(4): 917 - 921

BioMedSciDirect Publications

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International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com

Original Article

Interplay Of T Helper 1 and 2 Cytokines in Type 2 Diabetes Mellitus With and Without Microvascular Complications

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ARTICLE INFO

Keywords:
Interleukin 10,
Interferon gamma,
Cytokines,
Diabetes mellitus and
microvascular complications.

ABSTRACT

Background: Cytokines represent the major factors involved in the communication between thymus dependent T cells, macrophages and immune cells in the course of an immune response, the way and manner in which cytokines interact with each other could be a crucial factor in the pathogenesis of diabetic complications. The gluco – toxic microenvironment created in diabetes may influence the cell to cell signaling capabilities of cytokines. This study was designed to evaluate the pattern of interaction of T helper 1 and 2 cytokines in various micro vascular complications (MC) in Diabetes Mellitus. Methods: This was a cross sectional study carried out in 200 type2 Diabetes Mellitus (DM) and 100 sex and age matched healthy controls aged between 40 – 75 years. Type 2 DM patients were subdivided on the basis of presence or absence of MC. We determined plasma levels of interferon gamma (T helper 1 cytokine) and interleukin 10 (T helper 2 cytokine) in the study population using standard elisa technique. Statistical analysis used includes student's t test and analysis of variance. Results: Significant increase in the mean level of interleukin10 was observed in type 2 DM patients when compared with healthy controls (32.54 ± 1.67 pg/ml Vs 12.35 ± 1.60 pg/ml, p = 0.001), the concentration of which was observed to be much higher in DM with MC. The mean level of interferon gamma was significantly reduced in DM patients in comparison with controls (4.36 ± 0.63 pg/ml Vs 6.71 ± 0.43 pg/ml, p = 0.012), this value was lower in DM with MC. Conclusion: The interplay of cytokine thus expressed may suggest a possible role for these in the pathogenesis of MC and could introduce novel approach in therapeutics.

1. Introduction

The prevalence of diabetes mellitus has been increasing progressively, and the number of adults with diabetes in the world is projected to increase to 220 million in the year 2020 [1]. In Nigeria, it is the commonest endocrine condition that is seen in Medical outpatients' clinic. Here, approximately 5 million people live with the disease, the highest number in the African continent [2]. Some communities record prevalence as high as 5 – 10 % [3]. Chronic elevation of blood glucose leads to damage to blood vessels (angiopathy). The endothelial cells lining the blood vessels take in more glucose than normal, since they don't depend on insulin. They then form more surface glycoproteins than normal, and cause the basement membrane to grow thicker and weaker. In diabetes, the resulting problems are grouped under ''microsvascular disease'' (due to damage to small vessels) and ''macrovascular disease'' (due to damage to arteries). A number of serious microvascular complications such as retinopathy, neuropathy, nephropathy, foot ulcers, etc are a major cause of morbidity, hospitalization and mortality in type 2 diabetic Nigerians. The seriousness of diabetes mellitus is largely as a result of these associated complications, which can be severe, disabling and even fatal. Previous studies on prevalence of complications reported up to the early 1990s in sub Saharan Africa gave widely variable figures. The figures range from 9 – 16% for cataracts, 7 – 52% for retinopathy, 6 – 47% for neuropathy, 6 – 30% for nephropathy, 1 – 5% for macroangiopathy[4,5].

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Despite the progress made in elucidating the causes of diabetic microvascular complications, the possible implication of the innate immune system to this scenario has not been widely studied. Recent studies from Europe suggested immune abnormalities in type 2 diabetes mellitus6,7,8, but there was no concrete patho physiological evidence to elucidate the actual cause of this immune abnormality, especially with the complications that may arise. Dysregulation of the immune system may also be a contributing factor. The ability of cells to perceive and correctly respond to their microenvironment is the basis of development, tissue repair and immunity as well as tissue homeostasis. Cytokines are components of a large complex signaling network. The effects of cytokines on target cells may be inhibited or enhanced by other cytokines, hormones, cytokine receptor antagonists and circulating receptors9. Cytokines also mediates inflammatory response(especially the T helper 1 cytokines), which is a key component of the early immune response. Inflammatory response has been observed with people with type 2 diabetes mellitus10. This is the immune system's response to pathogens and tissue damage. While the inflammatory pathways of type 2 Diabetes have received much attention, the anti – inflammatory (T helper 2 cytokines) side of the equation is less well known. The extent and possible synergistic protective role of other cytokines in diabetics especially with regards to the complications that may arise may need to be investigated.

This study focuses on evaluating the pattern of cytokine immune response (produced by the helper T cells) with type 2 diabetes mellitus with and without microvascular complications

2. Materials and Methods

This was a cross sectional study carried over 3 months. The study population consisted of two hundred type 2 diabetes mellitus patients attending the diabetic clinics of Lagos State University Teaching Hospital, Lagos, Nigeria. These categories of patients were males and females between the ages of 40 and 60 years. Ethical approval was received from the Ethics and Research committee of the teaching hospital before commencement of the study. Informed consent of the patients was gotten through the aid of a well structured questionnaire. Patients excluded in this study were those on immunosuppressive drugs, systemic illnesses, those on steroids. The control samples were 100 apparently healthy age matched males and females. Fasting Blood Samples were collected into fluoride oxalate and lithium heparin bottles.

LABORATORY ANALYSIS: Fasting blood glucose was done spectrophotometrically using the glucose oxidase method11, while interleukin 10 and interferon gamma were estimated by standard elisa technique using IBL – hamburg kit, Germany. Intra and inter assay coefficient of variation of interleukin 10 was 3.2% and 5.6% respectively while intra and inter assay coefficient of variation of interferon gamma was 4.5% and 5.7% respectively. Long term glycaemic control (HBAIc) was assessed using chromatographic – spectophotometric ion exchange method.

3. Results:

The mean ± SEM level of interferon gamma was significantly lower in DM patients in comparison with controls (4.36 ± 0.63 Vs 6.71 ± 0.43 pg/ml, p = 0.012) while the mean ± SEM of interleukin 10 was significantly increased in DM patients in comparison with controls (32.54 ± 1.67 Vs 12.35 ± 1.60 pg/ml, p = 0.001). These cytokines were also found in lower and higher concentrations respectively in diabetic patients with microvascular complications in contrast to those without complications (see table 1). In this study, forty of them (20%) had retinopathy, sixty two (31%) had neuropathy, two patients (1%) had nephropathy with renal failure while fifteen of them (7.5%) had foot ulcers. A total number of eighty one (40.5%) was without any obvious complications. The pattern of cytokines observed in those with retinopathy showed significantly lower mean ± SEM levels of interferon gamma in comparison with those without any complications (2.53 ± 0.86pg/ml Vs 4.53 ± 1.06pg/ml, P= 0.042). Interleukin 10 was significantly elevated in diabetic patients with retinopathy in comparison with those without complications (42.50 ± 3.60pg/ml Vs 30.31 ± 2.63pg/ml, p = 0.048). Diabetic neuropathy reveal significant increase in interleukin 10 level in comparison with those without complications (36.87 ± 3.61pg/ml Vs 30.60 Vs 2.31pg/ml, p = 0.041), while interferon gamma level was also observed to be significantly reduced in comparison with those without complications (3.24 ± 1.22pg/ml Vs 4.60 ± 0.95pg/ml, p = 0.050). Diabetic patients with renal failure as a result of nephropathy showed insignificant differences in the mean levels of interleukin 10 (39.20 ± 7.22pg/ml Vs 30.60 ± 2.31pg/ml, p = 0.893) and interferon gamma (7.30 ± 6.60pg/ml Vs 4.53 ± 0.99pg/ml, p = 0.872) in comparison with those without complications. Diabetic patients with foot ulcers had significantly raised interleukin 10 levels (40.53 ± 10.21pg/ml Vs 30.60 ± 2.31pg/ml, p = 0.048) while insignificantly increased interferon gamma was observed (5.99 ± 4.52pg/ml Vs 4.23 ± 0.87pg/ml, p = 0.460) in comparison with DM patients without complications. The proportions of DM patients who attained good – long term glycaemic control was 68% respectively.

4.Discussion

The pattern of cytokine immune response in complicated and uncomplicated diabetics was evaluated. This was in a bid to assess their relative roles in organ complications resulting from diabetes mellitus. In this study, interleukin 10 was significantly higher in DM than the control group ($p < 0.05$). This cytokine was also found in higher concentrations in diabetic patients with microvascular complications in contrast to those without complications. A significant reduction in interleukin 10 especially in the healthy subjects may suggest a possible role for these cytokines in the pathogenesis of diabetic microvascular complications.

Interleukin 10 plays an important role as a regulator of lymphoid and myeloid cell function. This is produced by the helper 2 cells of the lymphocytes. Interleukin 10 suppresses the effector functions of the macrophages, T cells and natural killer cells. Increased levels of interleukin 10 in Nigerian diabetics may not be advantageous

after all due to its immunosuppressive properties. The immunosuppressive property of this cytokine has been of immense benefit in suppressing rejections of grafts after organ transplantations. But however, excessively high levels of this cytokine may account for the immuno suppression commonly encountered by diabetics. Also worthy of note in this study is significant reduction of interferon gamma observed in diabetic patients in comparison with healthy subjects ($p < 0.05$). This reduction was found to be more pronounced in complicated diabetes mellitus patients. Interferon gamma, otherwise known as type 11 interferon or immune interferon thus offered greater protection to the healthy subjects. Previous study corroborated the protective role of interferon gamma in which an increase in a T helper 1 cytokine (interferon gamma) ensured the survival of laboratory animals after contact with pathogens, whereas the predominance of T helper 2 cytokine (interleukin 10) led to a lethal course of infection12. The reduced interferon gamma observed in diabetic patients especially in those with complications may limit antibody formation due to the inability of interferon gamma to express major histocompatibility complex (MHC) antigens on macrophages, T cells and B cells.

The present study also focused on diabetic Nigerians already with frank and visible complications. Forty of them (20%) had retinopathy, sixty – two (31%) had neuropathy, two (1%) had nephropathy with renal failure while fifteen (7.5%) had foot ulcer. The pattern of cytokines observed in those with retinopathy showed significantly lower levels of interferon gamma ($p < 0.05$) in comparison with those without any complications. Interleukin 10 was significantly elevated ($p < 0.05$) in diabetic patients with retinopathy in comparison with those without complications. The eye has two major mechanisms to actively suppress cell mediated responses: the epithelial cells lining the anterior chamber express Fas ligand and the fluid of the anterior chamber contains cytokines such as transforming growth factor (TGF B). Fas is a molecule expressed on a variety of cells that acts as a target for ligation by Fas ligand on cytotoxic cells. Expression of Fas ligand on the epithelial cells permits engagement on incoming cells (T cells) that express Fas and thereby inducing their apoptosis (cell death). Additionally, expression of Fas ligand may also favour a switch to a T helper 2 type (interleukin 10) response because Fas ligand has been shown to induce death of T helper 1 cells (mainly interferon gamma) more easily than T helper 2 cells13. The glucotoxic microenvironment created in diabetes mellitus has been shown to affect Fas expression14. A similar trend in cytokine pattern was also observed in diabetic patients with neuropathy. Interferon gamma in diabetics with neuropathy was significantly lower compared with diabetic patients without complications ($p < 0.05$). More so, significant elevation of interleukin 10 was also observed in diabetic neuropathy in comparison with those without complications ($p < 0.05$). A plausible reason for this is as a result of switching mechanisms from the T helper 1 cells to the T helper 2 cells which also occur in the central nervous system. More intriguing results of cytokines were observed in diabetic patients with foot ulcers. This may be sequel to sensory neuropathic changes. Neuropathy is a major factor in the development of foot ulcers. Neuropathy makes the nerves either hypersensitive or

Table 1: means ± SEM of cytokines in diabetics with complications, diabetics without complications and control subjects.

Variables	Controls n = 100	Diabetics without compli-cations n = 81	Diabetics with compli cations n = 119	f value	p value
Cytokines (pg/ml)					
Interferon - gamma	6.71± 0.43	4.62 ± 0.98	4.03± 0.82	3.311	0.038 *
Interferon - gamma	12.35±0.60	29.38± 1.86	36.73± 2.98	40.15	0.000 *

Table 2: means± SEM of cytokines in diabetics with retinopathy in comparison with diabetics without complications.

Variables	Patients with retinopathy n = 40	Patients without complications n = 81	t value	p value
Cytokines (pg/ml)				
Interferon - gamma	2.53± 0.86	4.53± 1.06	1.682	0.042 *
Interleukin - 10	42.50± 3.60	30.31± 2.63	2.208	0.048 *

Table 3: means± SEM of cytokines in diabetics with neuropathy in comparison with diabetics without complications.

Variables	DM patients with neuropathy n = 62	DM patients without complications n = 81	t value	p value
Cytokines (pg/ml)				
Interferon - gamma	3.24± 1.22	4.60± 0.95	1.690	0.050 *
Interleukin - 10	36.87± 3.61	30.60± 2.31	3.602	0.041 *

Table 4: means± SEM of cytokines of diabetics with foot ulcers in comparison with diabetics without complications.

Variables	DM patients with food ulcers n = 15	DM patients without complications n = 81	t value	p value
Cytokines (pg/ml)				
INF - gamma	5.99± 4.52	4.23± 0.87	0.518	0.460
IL 10	40.53± 10.21	30.60± 2.31	1.972	0.048 *

Table 5: Logistic regression analysis of cytokines in T2 DM with good long term glycaemic control (HBA1c < 7%) and various microvascular complications.

Variables	Retinopathy			Neuropathy			Foot ulcers			Nephropathy		
	O.R	C.I	P	O.R	C.I	P	O.R	C.I	P	O.R	C.I	P
INF g	0.951	0.843/1.072	0.406	0.983	0.945/1.02	0.376	0.538	0.208/1.386	0.199	1.020	0.933/1.115	0.661
IL 10	1.011	0.991/1.031	0.274	1.003	20.986/1.02	0.713	0.97	0.929/1.01	0.185	1.00	0.948/1.06	0.885

OR – Odds ratio, CI – Confidence interval, P – Probability* < 0.05(Significant)

Table 6: Logistic regression analysis of cytokines in T2 DM with poor long term glycaemic control (HBA1c > 7%) and various microvascular complications.

Variables	Retinopathy			Neuropathy			Foot ulcers			Nephropathy		
	O.R	C.I	P	O.R	C.I	P	O.R	C.I	P	O.R	C.I	P
INF g	1.047	0.963/1.138	0.283	0.8036	0.673/0.959	0.015*	0.976	0.858/1.110	0.709	1.07	0.000/38.3	0.999
IL 10	0.317	0.101/0.995	0.048*	1.012	0.998/1.026	0.042*	1.058	1.006/1.113	0.027*	0.968	0.000/1.05	0.999

O.R – Odds ratio, C.I – Confidence interval, P – Probability

hyposensitive leading to a foot which has impaired ability to recognize pain, pressure, temperature differences and position. The overall effect is a leg or foot prone to trauma or high external pressure. Interleukin 10 levels were observed to be significantly elevated when compared with those without complications ($p < 0.05$). Excessively high interleukin 10 levels observed in diabetics with foot ulcers may account for increased susceptibility to infections in these patients as a result of the immunosuppressive property of the cytokine. The common initiating infection may be a fungal infection which thereafter becomes secondarily infected with bacteria. Such infections often progress spreading along the tissue planes till overwhelming limb and life threatening cellulitis and septicaemia ensue. A steep rise in interferon gamma observed in this regard is in contrast to the pattern noticed in other complications. Could this suggest that serum levels of interferon gamma may not drop beyond certain limit? Or could it be that another mechanism is at work? A plausible explanation could be due to the fact that interferon gamma is stimulated to fight against viruses and bacteria which are causative agents in most ulcers. Viruses and many bacteria can replicate at an enormous rate and potentially have the capacity to overwhelm the individual before the adaptive immune system gets going. It takes several days to activate and expand clones of antigen specific lymphocytes. During this time, interferon gamma and natural killer cells are particularly important in slowing the spread of infection. One other possible explanation could be due to the involvement of interferon gamma in the phases of wound healing15. This includes; homeostasis, inflammation, cellular migration and proliferation, Protein synthesis (collagen) and wound contraction and remodeling. Remodelling begins to predominate as the primary wound healing activity approximately twenty one days after injury. The rate of collagen synthesis diminishes and reaches coincidence with rate of collagen breakdown. The down regulation of collagen synthesis is mediated by interferon gamma16. The fact that interferon gamma was low in those with neuropathy which is sequel to development of foot ulcers is an indication of reduced protection offered by interferon gamma in diabetic neuropathy. Significant increase in interleukin 10 levels in nephropathy had been reported17. Insignificant differences in level of these cytokines in patients with renal failure was observed in this study (interleukin 10 -39.20 ± 7.22pg/ml Vs 30.60 ± 2.31pg/ml, p=0.893 and interferon gmma – 7.30 ± 6.60pg/ml Vs 4.53 ± 0.99 pg/ml, p = 0.872). Lower incidence in this category may have affected this pattern.

Logistic regression analysis was carried out to evaluate the possibility of using cytokines in predicting the future development of microvascular complications in T 2 DM patients yet to have microvascular complications. This was assessed in T2 DM with good long term glycaemic control (HBA1c < 7%) as well as in those with poor long term glycaemic control (HBAIc >7%). The independent variables entered into the model included the cytokines, while the various microvascular complications served as the dependent variables. In this study neither of the cytokines predicted microvascular complications in T2 DM with good glycaemic control, but were found to be predictors of microvascular complications in T2 DM with poor glycaemic control. In this subset, increased levels of interlukin 10 predicted retinopathy. Increased interleukin 10 levels predicted the occurrence of neuropathy and foot ulcers while decreased levels of interferon gamma predicted neuropathy only.

5. Conclusion

Dysregulation of a T helper 1 cell (interferon gamma) and a T helper 2 cell (interleukin – 10) are implicated in the pathogenesis of diabetic mirovascular complications. Increased interferon gamma and decreased interleukin – 10 concentrations may delay development of microvascular complications in T2 DM

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