ORIGINAL ARTICLE

ASSOCIATIONS AMONG SERUM GLUCOSE AND INSULIN WITH PRO-INFLAMMATORY AND ANTI-INFLAMMATORY CYTOKINE LEVELS IN BANGLADESHI NEWLY DIAGNOSED TYPE 2 DIABETES MELLITUS PATIENTS

Khadija Akhter Jhuma[1], Abu SM Giasuddin[2], Md. Sabir Hossain[3]

[1]Department of Biochemistry & Molecular Biology, Medical College for Women & Hospital (MCW&H) & Member Secretary of Medical Research Unit (MRU), Medical & Health Welfare Trust (MHWT), Uttara, Dhaka

[2]Department of Biochemistry & Immunology and Director of Medical Research Unit (MRU), MHWT, MCW&H, Uttara, Dhaka

[3]Department of Biochemistry & Molecular Biology, Jahangirnagar University, Savar

ABSTRACT

Literature review indicated that very limited studies on associations (correlations) among serum glucose and insulin with pro-inflammatory (IL-1, IL-6, TNF-α) and anti-inflammatory cytokine (IL-4, IL-10, Il-13) levels in newly diagnosed Bangladeshi patients with Type 2 dibetes millitus (T2DM) have been reported. We therefore investigated those associations in newly diagnosed Bangladeshi T2DM patients and the findings are reported in the present article. Suspected patients with T2DM had undergone oral glucose tolerance test (OGTT) after overnight fasting. Blood samples were collected from patients at '0' min (Fasting) and at 2 hrs (120 min) after OGTT. Newly OGTT positive 36 adult patients (Male: 15, Female: 21, Age: 35-65 years) and 30 normal healthy subjects (Male: 12, Female: 18; Age range: 32-67 years) were investigated in parallel as controls. Patients having any kind of metabolic diseases and taking hypoglycemic drug therapy were excluded from the study. Serum levels of pro–inflammatory (IL-1, IL-6, TNF-α) and anti-inflammatory cytokines (IL-4, IL-10, Il-13) were determined by enzyme immunoassay (EIA) methods using research kits obtained from reputed companies at Medical Research Unit (MRU), MCW&H building, MHWT, Dhaka, Bangladesh. The associations among FBG and fasting pro-inflammatory cytokines (F-IL-1, F-IL-6, F-TNF-α) revealed that F-IL-1 vs F-TNF-α ($r=0.346$, $p=0.039$) and F-IL-1 vs F-IL-6 ($r=0.394$, $p=0.018$) were significant ($p<0.05$). But, FBG level did not show significant correlations with any of the pro-inflammatory cytokines ($p>0.05$). The associations of pro-inflammatory cytokines at 2 hrs of OGTT (IL-1, 2hr, IL-6, 2hr, TNF-α, 2hr) in T2DM patients revealed that IL-1, 2hr vs TNF-α, 2hr was significant ($r=0.454$, $p=0.005$). Blood glucose 2 hours (BG2Hr) level did not show significant correlations with any of the pro-inflammatory cytokines ($p>0.05$). Associations of F-insulin with any of the fasting pro-inflammatory cytokines were not significant ($p>0.05$). However, the associations of F-IL-1 vs F-IL-6 ($r=0.394$, $p=0.018$) and F-IL-1 vs F-TNF-α ($r=0.346$, $p=0.039$) were significant ($p<0.05$). The associations for insulin 2hr, with any of the pro-inflammatory cytokines were not significant ($p>0.05$). However, IL-1, 2hr vs TNFα, 2hr was associated significantly ($r=0.454$, $p=0.005$). FBG did not show any significant associations with anti-inflammatory cytokines ($p>0.05$). BG2Hr also did not show any

significant associations with the anti-inflammatory cytokines ($p > 0.05$). However, the association between IL-4, 2Hr vs IL-13, 2hr was significant ($r = 0.380$, $p = 0.022$). F-Insulin and any of the other comparisons did not show any significant associations ($p > 0.05$). Also, Insulin 2Hr did not show any significant associations ($p > 0.05$). However, the association between IL-4, 2Hr vs F-IL-13, 2Hr was found to be significant ($p = 0.022$). These significant and non-significant associations obtained among FBG, BG2Hr, F-Insulin and insulin 2hr with pro-inflammatory (IL-1, IL-6, TNF-α) and anti-inflammatory (IL-4, IL-10, IL-13) cytokines were discussed relevant to pathogenesis of T2DM in Bangladeshi patients.

Key words: T2DM, Glucose, Insulin, Cytokine, Pro-inflammatory, Anti-inflammatory

Introduction

Diabetes mellitus (DM) poses a major global health threat, both in the developed and developing countries with type 2 diabetes mellitus (T2DM) accounting for about 90-95% of all cases[1-3].

It seems to be useful to know the levels of various cytokines, a heterogenous group of protein cell regulators, in blood and other body fluids in order to monitor disease activity or monitor treatment or evaluate the need for treatment of T2DM[4,5]. However, conflicting and contrasting results of increase in postprandial plasma IL-6 and TNF-α levels were reported in T2DM patients[6,7]. The involvement of pro-inflammatory cytokines was substantiated by the observation that IL-1 blockade attenuates β-cell dysfunction by islet amyloid-induced inflammation in T2DM. It has been reported that TNF-α induced insulin resistance is associated with an elevated expression of IL-10 in human skeletal muscle tissue, which suggests a possible role for IL-10 in the pathogenesis of TNF-α induced insulin resistance in humans. Also, it has been reported that anti-inflammatory cytokines (IL-4, IL-10, IL-13, etc.) counteract the cytotoxic effects of pro-inflammatory cytokines, i.e., IL-1, IL-6, TNF-α, etc. in insulin-producing cells. Thus, a balance between the anti-inflammatory and the pro-inflammatory cytokines is of crucial importance for the prevention of pancreatic β-cell destruction. It is therefore evident that perturbation of this delicate balance in favour of pro-inflammatory cytokines is a strong possibility as pathogenetic mechanism towards development of T2DM[5-7].

As indicated by literature review that very limited studies on balance between pro-and anti-inflammatory cytokine levels have been reported in T2DM patients from Bangladesh, we conducted a case-control prospective interventional study and reported the results on the status of pro-inflammatory (IL-1, IL-6, TNF-α,) and anti-inflammatory (IL-4, IL-10, IL-13) cytokines in newly diagnosed Bangladeshi patients with T2DM[8]. These results reported for the first time in Bangladeshi patients with T2DM supported the concept that T2DM possibly develops due to imbalance among

pro-inflammatory and anti-inflammatory cytokines. We have extended the study results looking into the associations (correlations) among various factors in our patients. In the present article, therefore, the associations (correlation) among serum status of glucose and insulin with status of serum pro-inflammatory (IL-1, IL-6, TNF-α) and anti-inflammatory (IL-4, IL-10, IL-13) cytokines in newly diagnosed Bangladeshi patients with T2DM have been reported.

Materials and Methods

This case-control prospective interventional study was done in Medical Research Unit (MRU), MCW & H Building, MHWT, Uttara, Dhaka, Bangladesh. As reported previously, newly diagnosed Bangladeshi patients with T2DM were included in the study. Suspected patients with T2DM had undergone oral glucose tolerance test (OGTT) after overnight fasting. Blood samples were collected from individual patients at 'O' min (Fasting) and at 2 hours (120 min) after OGTT[8]. Samples were aliquoted for routine analyses and special research investigations. Among the routine analyses, glucose level and other usual routine tests in blood, serum and urine were done. Serum aliquots were preserved for a short time at -20^{O}C to -80^{o}C for hormones, cytokines and other special investigations. A total of 36 OGTT positive adult patients (male: 15, female: 21, age range 35-65 years; mean±SD: 47±18 years) were included in this case-control prospective study. And 30 normal healthy adults (male: 12, female: 18, age range 35-65 years; mean±SD: 47±18 years) were investigated in parallel as normal control subjects. Suspected patients with T2DM underwent oral glucose tolerance test (OGTT) after overnight fasting maintaining all procedures; aliquots of 7-10 mL blood samples were collected from individual patients at 'O' min (Fasting) and 2 hours (120 min) after giving 75 gm glucose in 300 mL water orally. Serum samples were aliquoted in separate microcentrifuge tubes and stored frozen at -80^{0}C deep freezer. Hormones and cytokine analyses were done in batches after collection from T2DM patients and normal controls. Pationts with Comonbidities, such as thyroid diseases, any endocrine disorders, renal diseases, and hypertension were excluded.

Routine laboratory investigations such as blood glucose, CBC, HbA1c, LFTs, TFTs, RFTs were done adopting usual clinical laboratory methods as practiced in the hospital laboratory. The special investigations, i.e; serum levels of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) and anti-inflammatory cytokines (IL-4, IL-10, IL-13) were done by adopting enzyme immunoassay (EIA) methods using kits obtained from reputed commercial companies such as R&D Systems (USA), Calbiotech (USA), Novatech (Germany)[9]. Statistical analyses were performed by various tests including multiple correlation coefficient test for associations (correlations) using SPSS programed in computer[10].

Results

The associations in T2DM patients among FBG and fasting pro-inflammatory cytokines (F-IL-1, F-IL-6, F-TNF-α) are stated in Table I. The analysis on the associations among FBG with fasting pro-inflammatory cytokines (F-IL-1, F-IL-6, F-TNF-α) revealed that the results of F-IL-1 Vs F-TNF-α ($r=0.346$, $p=0.039$) and F-IL-1 vs F-IL-6 ($r=0.394$, $p=0.018$) were significant ($p<0.05$). But, FBG level did not show significant correlations with any of the pro-inflammatory cytokines ($p>0.05$).

Table I: Associations among FBG and pro-inflammatory cytokines (F-IL-1, F-IL-6, F-TNFα) in T2DM patients

		FBG (mmol/L)	F- IL-1 (ng/L)	F-IL-6 (ng/L)	F-TNF -α (ng/L)
FBG (mmol/L)	r value	1	.213	-.109	-.044
	p value		.213	.525	.800
	N	36	36	36	36
F- IL-1 (ng/L)	r value	.213	1	.394 *	.346 *
	p value	.213		.018	.039
	N	36	36	36	36
F-IL-6 (ng/L)	r value	-.109	.394 *	1	.120
	p value	.525	.018		.486
	N	36	36	36	36
F-TNF -α (ng/L)	r value	-.044	.346 *	.120	1
	p value	.800	.039	.486	
	N	36	36	36	36

*Correlation is significant at the 0.05 level (2-tailed)

FBG: Fasting Blood Glucose; F-IL-1: Fasting IL-1; F-IL-6: Fasting IL-6; F-TNF-α: Fasting TNF-α; **$P<0.05$: significant, $P>0.05$: not significant

The associations among BG2Hr and pro-inflammatory cytokines (IL-1,2hr, IL-6,2hr and, TNFα 2hr) in T2DM patients are stated in Table II. The analysis on the associations of pro-inflammatory cytokines at 2 hours of OGTT (IL-1, 2Hr, IL-6, 2Hr, TNF-α, 2Hr) in T2DM patients revealed that IL-1, 2Hr vs TNF-α, 2Hr ($r=0.454$, $p=0.005$) were significant. BG 2Hr level did not show significant correlations with any of the pro-inflammatory cytokines ($p>0.05$).

Table II: Associations among BG2Hr and pro-inflammatory cytokines (IL-1, 2hr, IL-6, 2Hr and TNF-α, 2Hr) in T2DM patients

		BG2Hr (mmol/L)	IL-1,2Hr (ng/L)	IL-6,2Hr (ng/L)	TNF-α, 2Hr (ng/L)
BG 2Hr (mmol/L)	r value	1	.181	.047	.083
	p-value		.291	.786	.630
	N	36	36	36	36
IL-1,2Hr (ng/L)	r value	.181	1	.072	.454**
	p value	.291		.675	.005
	N	36	36	36	36
IL-6,2Hr (ng/L)	r value	.047	.072	1	.115
	p value	.786	.675		.506
	N	36	36	36	36
TNF-α, 2Hr (ng/L)	r value	.083	.454**	.115	1
	p value	.630	.005	.506	
	N	36	36	36	36

**Correlation is significant at the 0.01 level (2-tailed).

BG2Hr: Blood Glucose at 2Hr of OGTT; IL-1, 2Hr: IL-1 at 2Hr of OGTT; IL-6, 2Hr: IL-6 at 2Hr of OGTT; TNF-α, 2Hr: TNF-α, at 2Hr of OGTT; ***P<0.05: significant, P>0.05: not significant*

The associations among F-Insulin and pro-inflammatory cytokines (F-IL-1, F-IL-6, and F-TNF-α) are stated in Table III. Associations F-insulin with any of the fasting proinflammatory cytokines were not significant ($p > 0.05$). However, the associations of F-IL-1 vs F-IL-6 ($r = 0.394$, $p = 0.018$) and F-IL-1 vs F-TNF-α ($r = 0.346$, $p = 0.039$) were significant ($p < 0.05$).

Table III: Associations among F-Insulin and pro-inflammatory cytokines (F-IL-1, F-IL-6, F-TNFα) in T2DM patients

		F-Insulin (μIU/mL)	F- IL-1 (ng/L)	F-IL-6 (ng/L)	F-TNF -α (ng/L)
F-Insulin (μIU/mL)	r value	1	.160	-.261	.052
	p value		.350	.124	.763
	N	36	36	36	36
F- IL-1 (ng/L)	r value	.160	1	.394 *	.346 *
	p value	.350		.018	.039
	N	36	36	36	36
F-IL-6 (ng/L)	r value	-.261	.394 *	1	.120
	p value	.124	.018		.486
	N	36	36	36	36
F-TNF -α (ng/L)	r-value	.052	.346 *	.120	1
	p value	.763	.039	.486	
	N	36	36	36	36

*Correlation is significant at the 0.05 level (2-tailed).

F-Insulin: Fasting Insulin; F-IL-1: Fasting IL-1; F-IL-6: Fasting IL-6; F-TNF-α: Fasting TNF-α; *$P < 0.05$: significant, $P > 0.05$: not significant

The associations among insulin 2Hr and pro-inflammatory cytokines (IL-1, 2Hr, IL-6, 2hr, TNFα, 2hr) are stated in Table IV. The associations for Insulin 2Hr, with any of the proinflammatory cytokines were not significant ($p > 0.05$). However, IL-1, 2Hr vs TNF-α, 2Hr was associated significantly ($r = 0.454$, $p = 0.005$).

Table IV: Associations among insulin 2Hr and pro-inflammatory cytokines (IL-1, 2Hr, IL-6, 2Hr, TNF-α, 2Hr)) in T2DM patients

		Insulin2Hr (μIU/mL)	IL-1,2Hr (ng/L)	IL-6,2Hr (ng/L)	TNF-α, 2Hr (ng/L)
Insulin2Hr (μIU/ml)	r value	1	.268	-.143	.072
	p value		.115	.407	.677
	N	36	36	36	36
IL-1,2Hr (ng/L)	r value	.268	1	.072	.454**
	p value	.115		.675	.005
	N	36	36	36	36
IL-6, 2Hr (ng/L)	r value	-.143	.072	1	.115
	p value	.407	.675		.506
	N	36	36	36	36
	r value	.072	.454**	.115	1

**Correlation is significant at the 0.01 level (2-tailed).

Insulin2Hr: Insulin at 2Hr of OGTT; IL-1, 2Hr: IL-1 at 2Hr of OGTT; IL-6: 2Hr: IL-6 at 2Hr of OGTT; TNF-α, 2Hr: TNF-α at 2Hr of OGTT; ***P < 0.05: significant, P > 0.05: not*

Associations among FBG and anti-inflammatory cytokines (F-Il-4, F-IL-10, F-IL13) in T2DM patients are stated in Table V. FBG did not show any significant associations, $p > 0.05$).

Table V: Associations among FBG and anti-inflammatory cytokines (F-IL-4, F-IL-10, F-IL-13) in T2DM patients

		FBG (mmol/L)	F-IL-4 (ng/L)	F-IL-10 (pg/mL)	F-IL-13 (pg/mL)
FBG (mmol/L)	r value	1	-.061	-.078	.092
	p value		.725	.652	.592
	N	36	36	36	36
F-IL-4 (ng/L)	r value	-.061	1	.043	.293
	p value	.725		.805	.082
	N	36	36	36	36
F-IL-10 (pg/mL)	r value	-.078	.043	1	.055
	p value	.652	.805		.752
	N	36	36	36	36
F-IL-13 (pg/mL)	r value	.092	.293	.055	1
	p value	.592	.082	.752	
	N	36	36	36	36

Correlation is significant at the 0.05 level (2-tailed)

*FBG: Fasting Blood Glucose; F-IL-4: Fasting IL-4; F-IL-10: Fasting IL-10; F-IL-13: Fasting IL-13; ***P < 0.05: significant, P > 0.05: not significant*