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Association of IL-6 & IL-1β (pro-inflammatory cytokines) and related biochemical indexes in newly diagnosed diabetics subjected to glucose tolerance test

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Abstract: The study proposed to find out the association of pro-inflammatory cytokines (IL-6 & IL-1β) and related biochemical indexes in newly diagnosed diabetes (NDD) subjects as compared to healthy subjects. This clinical prospective research was done with collaboration of University of Karachi and Baqai Institute of Diabetology and Endocrinology between November 2018 to May 2019. Demographics and anthropometric details were noted on predesigned questionnaire. Subjects were identified on the basis of Oral Glucose Tolerance Test (OGTT). Samples of blood at baseline were gained for IL-6 & IL-1β (pro-inflammatory cytokines) and related biochemical indexes. Total of 34 subjects were included both males 19 (55.9%) and females 15 (44.1%) having mean age 49.65±1.95 years. On the basis of OGTT, 17(50%) were healthy subjects and 17(50%) were NDD. Mean ± SE value of IL-1β was 208.56±23.53 in healthy subjects and 1510.47±494.16 in NDD subjects, while, IL-6 was 57.51±13.02 and 119.51±36.60, respectively. Non-significant correlation was observed between IL- 6 and IL- 1β (r= 0.20, P=0.475) among healthy subjects. While, significant correlation was observed between IL- 6 and IL- 1β (r=0.774, P<0.0001) among NDD subjects. With increased levels of both IL-6 and IL-1β in NDD subjects only IL-1β showed significant correlation as compared to IL-6. In addition, significant correlation of IL-1β with various biochemical parameters as compared to IL-6 were also observed to be involved in progression from normoglycemia to type 2 DM.

Keywords: NDD, IL-6, IL-1β, biochemical indexes.

INTRODUCTION

Diabetes Mellitus Type 2 (T2DM**)** is a systemic disorder characterized by chronic hyperglycemia. It results via dynamic loss of β-cell insulin secretion and/or insulin obstruction (Hurtado and Vella, 2019). T2DM has number of risk factors related to medical condition, dietary intervention, lifestyle, environmental and psychosocial factors. During last decades the global rise in obesity, ageing population, sedentary lifestyles and physical inactivity in genetically predisposed individuals have magnified the incidence and prevalence of T2DM (Bellou *et al*., 2018; Aamir *et al*., 2019).

Chronic and systemic inflammation accompanying by circulating and dysregulated inflammatory biomarkers are the prominent features of T2DM. Inflammatory biomarkers are associated with hypercoagulable state and vascular dysfunction in T2DM, which in turn increases the risk of cardiovascular diseases (Randeria *et al*., 2019). The occurrence of IL-6 in tissues is a normal consequence, but elevated circulating levels of these inflammatory markers are considered as an independent predictor of T2DM which is involved in inflammation, IR and β-cell dysfunction (Akbari and Hassan-Zadeh 2018).

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The bimodal upshot of IL -1β on β- cell mass and secretion of insulin by Zhao *et al* was also presented as twofold: i.e. low and high concentration of IL-1β (Zhao *et al*., 2014). β-cell proliferation was stimulated by low IL-1β levels that enhances glucose stimulation by secreting insulin and inhibiting apoptosis of β- cell. While, opposite effects were seen for increase IL- 1β concentration. Its contribution was also seen in both β-cell compensation and failure phase, enhancement of apoptosis of β-cell, diminishing proliferation of β- cell and mass in T2DM (Eguchi and Nagai, 2017). Currently, it is not known that in progression of T2DM these inflammatory responses play a primary role or produce secondary effect.

Symptoms in T2DM subjects may take many years to give the impression or be recognized, so many people remain unaware for a long time of their illness, until blood glucose in excess amount damaged the organs of body and complications of diabetes developed. Hyperglycemia in T2DM increases the risk of microvascular complications, whereas dyslipidemia is a major cause for macrovascular complications mortality (Hirano, 2018). Hypertension with diabetes is a strong risk factor for heart failure, atherosclerotic cardiovascular disease and microvascular complications. Reduced morbidity in multiple high-quality randomized controlled trials have been observed with reduction of elevated blood pressure in people with diabetes (De Boer *et al*., 2017).

These pathophysiological markers of T2DM depending on number and stage of complications produce significant disruption in organs functioning and body systems that consequently affect life quality. In our information, no such data for newly diagnosed diabetes type 2 (NDD) exists in our population showing association between serum liver biomarkers, deranged lipid profile, obesity and hypertension with pro-inflammatory cytokines. Hence, this study was proposed to find out association of pro-inflammatory cytokines (IL- 6 & IL- 1β) with related biochemical indexes in NDD subjects as compared to healthy subjects.

MATERIALS AND METHODS

This interventional clinical research was prospectively carried out with the collaboration of Biochemistry Department, Karachi University and Baqai Institute of Diabetology and Endocrinology (BIDE) in Pakistan. The study duration was from March 2019 to May 2019. Ethical approval was attained from Review committee of BIDE (IRB no.: BIDE/ IRB/NWARIS/10/26/18/0206) and experimental procedures and study purpose was described to each subject prior to taking informed written consent.

Both males and females aged between 30 to 70 years were included. Identification of subjects was done on the basis of OGTT according to world health organization criteria (World health organization, assessed 2020). Each subject was given Trelan G, 75-g glucose (specified anhydrate glucose water) for post glucose load. Subjects on the basis of OGTT were categorized as healthy subjects without diabetes [FPG (plasma fasting glucose) below 100 (mg/dL) and 2-hr PGL (2- hour post level glucose) below 140 (mg/dL)] and subjects with NDD [FPG ≥126(mg/dL) or 2-hr PGL ≥200 (mg/dL) or both] without using any anti diabetic medications (Basit *et al*., 2018; World health organization, assessed 2020). Individuals who do not agree to participate, with impaired plasma glucose, pregnancy, receiving anti-diabetic agents, receiving instructions on healthy diet and exercise from healthcare professionals, cerebrovascular, microvascular, macrovascular disease, severe renal dysfunctions, severe hepatic dysfunction, rheumatoid arthritis, osteoarthritis and any kind of allergies were excluded. Details of demographic and anthropometric measurements were noted on predesigned questionnaire. Blood samples at baseline were obtained for IL-6 & IL-1β (pro-inflammatory cytokines) with related biochemical indexes. Following inclusion criteria, biochemical indexes including FPG, 2-hr PGL, HbA1c, lipid profile, serum creatinine, AST (aspartate transaminase) and ALT (alanine transaminase) values were analyzed at laboratory of BIDE-Pakistan. While, blood samples for IL-6 & IL-1β were measured at University of Karachi-Pakistan following laboratory standard operating procedure.

Glucose (plasma level) was measured by oxidase peroxidase glucose technique and HbA1c by high-performance liquid chromatography phenomena. Serum cholesterol was measured using oxidase phenol 4-amino antipyrine peroxidase cholesterol method serum triglycerides using glycerol phosphate oxidase-p-amino phenazone process, serum HDL (lipoprotein high density)-cholesterol by enzymatic calorimetric homogeneous technique (Direct Method) and direct method was also used for measuring serum LDL (lipoprotein low density)-cholesterol. Serum ALT and AST were determined by modified International Federation of Clinical Chemistry method, serum creatinine by Erba Transasia autoanalyzer (XL-600) modified Jaffe's kinetic method. Serum pro-inflammatory cytokines IL-1β and IL6 were quantitatively evaluated by ELISA (enzyme linked immune-sorbent assay) kits method catalogue number E0143Hu and E0090Hu, respectively.

Height and bodyweight were measured using standardized techniques to compute BMI (body mass index). Obesity in term of BMI was considered 25 (kg/m^2) or higher for both males and females with or without abdominal obesity as explicated by guidelines of Asia Pacific or world health organization (Lim *et al*., 2017). Hypertension was detailed if blood pressure systolic was ≥140(mmHg) and/or blood pressure diastolic was ≥90(mmHg) on two different occasions and 15 minutes apart after been comfortable (Basit *et al*., 2020).

STATISTICAL ANALYSIS

SPSS (statistical package for social sciences) version 22 was used for analysis of data statistically. Variables (continuous) are reported as Mean ± Standard Error. Variables (categorical) are described as frequency and percentage. Correlation Spearman's rho was used. Significance value was mentioned as P-value<0.05 statistically.

RESULTS

Total of 34 subjects were included males 55.9% (19) and females 44.1% (14). Age in mean of the participants was 49.65±1.95 years. Mean ± SE of various biochemical parameters are presented in table 1. On the basis of OGTT, 17(50%) were healthy subjects and 17(50%) were NDD. Mean ± SE value of IL-1β was 208.56±23.53 in healthy subjects and 1510.47±494.16 in NDD subjects, while, IL-6 was 57.51±13.02 and 119.51±36.60, respectively.

Fig. 1 shows the correlation non-significantly (r= 0.20, P= 0.475) for IL-1β and IL-6 among healthy subjects. While, significant correlation (r= 0.774, P<0.0001) was observed between IL-1β and IL-6 among NDD subjects (fig. 2).

Table 1: Parameters at baseline of study participants

Variables	Mean ± SE or n (%)
n	34
Male	19 (55.9%)
Female	15 (44.1%)
Age (years)	49.65±1.95
Body mass index (kg/m^2)	28.12±0.93
Systolic blood pressure (mmHg)	117.56±1.98
Diastolic blood pressure (mmHg)	79.24±1.52
Fasting plasma glucose (mg/dl)	118.32±5.08
2-hour post glucose level (mg/dl)	165.92±15.98
Total cholesterol (mg/dl)	190.91±8.38
Triglyceride (mg/dl)	175.59±16.82
Lipoprotein low density (mg/dl)	120.44±7.4
Lipoprotein high density (mg/dl)	32.91±2.82
HbA1c (mg/dl)	6.52±0.25
Serum creatinine (mg/dl)	1.06±0.04
Aspartate transaminase (mg/dl)	31.3±2.8
Alanine transaminase (mg/dl)	26.24±3.49
IL -1β (pg/L)	880.51±277.94
IL -6 (ng/L)	89.51±20.39

Table 2: IL -1β and IL -6 correlation with several biochemical parameters

Parameters	Healthy subjects (n=17)				Newly diagnosed diabetes subjects (n=17)			
	IL -1β		IL -6		IL -1β		IL -6	
	r	P -value	r	P -value	r	P -value	r	P -value
Age	-0 .394	0.147	-0.236	0.397	0.13	0.632	0.249	0.352
Gender	0 .124	0.66	0.433	0.107	-0.196	0.467	-0.056	0.837
BMI	0 .104	0.713	-0.073	0.795	-0.276	0.30	-0.282	0.289
Systolic	0 .216	0.439	0.162	0.564	0.483	0.051	0.094	0.73
Diastolic	0.661	0.007	0.346	0.206	0.153	0.573	-0.11	0.684
FPG	-0.269	0.332	-0.129	0.646	0.486	0.057	0.345	0.19
2-hr PGL	0.024	0.935	0.266	0.358	0.285	0.458	-0.126	0.748
Cholesterol	0.011	0.97	-0.007	0.98	0.591	0.016	0.441	0.087
Triglyceride	0.054	0.85	0.239	0.39	0.447	0.083	0.032	0.905
LDL	0.389	0.152	-0.086	0.761	0.481	0.050	0.311	0.242
HDL	-0.011	0.97	0.054	0.849	0.383	0.144	0.483	0.051
HbA1c	-0.181	0.519	-0.158	0.575	0.246	0.359	0.027	0.922
Serum creatinine	-0.368	0.177	-0.072	0.799	0.215	0.424	-0.093	0.733
AST	0.068	0.809	-0.018	0.949	-0.318	0.248	-0.316	0.251
ALT	0.043	0.879	-0.23	0.409	-0.099	0.716	-0.172	0.523

Correlation Spearman's rho was used;
Significance levels considered as P -value<0.05

IL- 1β and IL- 6 correlation with various biochemical parameters are presented in table 2. Among healthy subjects correlation significantly found between IL- 1β and several biochemical parameters such as blood pressure (diastolic) (r= 0.661, P=0.007) and for IL-6 it was insignificant with all biochemical indexes. Among NDD subjects, significant correlation was observed between IL-1β and blood pressure (systolic) (r= 0.483, P= 0.051), FPG levels (r= 0.486, P= 0.057), cholesterol total (r= 0.591, P= 0.016) and cholesterol-LDL (r= 0.481, P= 0.050). For IL-6, significant correlation was only observed with cholesterol-HDL (r=0.483, p=0.051) in NDD subjects.

DISCUSSION

Increased IL -6 and IL -1β values were observed in NDD subjects in this research, while significant correlation was only detected for IL -1β in NDD subjects as compared to normal glucose subjects. Our results are consistent to most of the preceding researches that found improved levels of IL -6 and IL -1β in T2DM subjects (Sattar *et al*.,

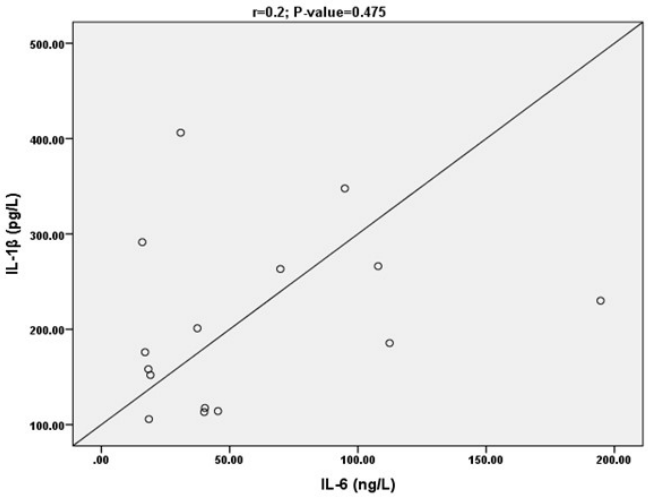

Fig. 1: IL -1β and IL -6 correlation among healthy subjects

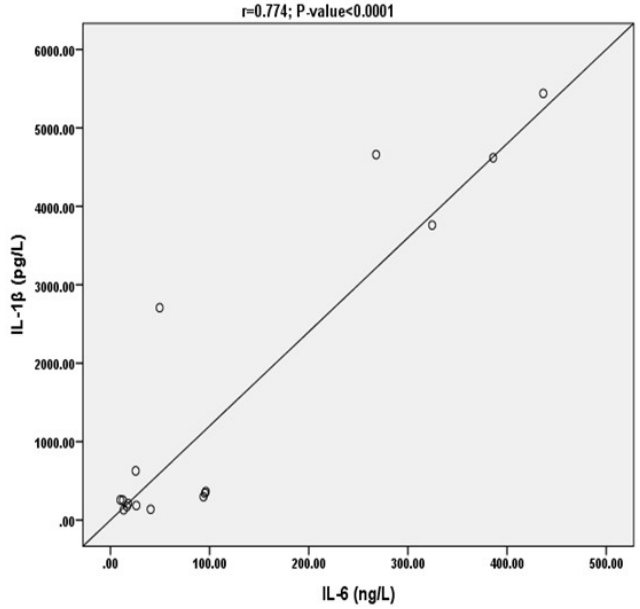

Fig. 2: IL -1β and IL -6 correlation among diabetic subjects

2018; Memon *et al*., 2013). Regarding cytokines and resistance of insulin many researches have been done globally. It was well known that higher levels of cytokines are found in diabetic subjects than normal individuals and this elevation might be interrelated to amplified oxidative stress, macrophages activation or induction of cytokines (Shelbaya *et al*., 2012). Our current outcomes are similar with Wang *et al* study showing elevated inflammatory cytokines in T2DM early stage and involved in determining the T2DM occurrence (Wang *et al*., 2016). Our data showed no such significant association for various biochemical parameters with IL-6 and IL-1β in healthy subjects, while IL -1β with several biochemical parameters showed correlation significantly that indulges systolic blood pressure, FPG, cholesterol (total) and LDL in NDD subjects. In NDD subjects, it indicates the multipart connections between cardiovascular factors and inflammatory cytokines. Previous studies showed that IL-6 is positively associated with BMI and hypertension in T2DM conversely to our study where significant results were only observed between IL-6 and HDL in NDD subjects (Lukic *et al*., 2014). However, our results are consistent to Mohammadi M *et al* study that showed no significant association between IL-6 and various metabolic syndrome components (Mohammadi *et al*., 2017). IL -6 with its co-inducible property might develop obesity-related IR in progress of T2DM, in contrast, no significant association exist between IL-6 and BMI in our findings.

In tissues, IL -1β endorse IR and is closely linked with mechanisms of atherosclerosis and inflammation related to obesity (Besedovsky and Rey, 2014). Under physiological conditions, IL-1β also play an important

role in metabolism of lipids by regulating insulin levels and lipase activity. We also found increased levels of IL-1β and significant association with total cholesterol, LDL and BMI consistent to previous study (Manica-Cattani *et al*., 2010; Wadood *et al*., 2014). These results opposed to Li *et al* study who observed no changes in IL-1β levels in NDD subjects (Li and Shen, 2019). Literature also suggest that in the liver regeneration process specific cytokines such as tumor necrosis factor-α and IL -6 play a vital role (Chae *et al*., 2018). Previous study also examined the correlations between biochemical markers of liver illness and different cytokines levels of serum (Mourtzikou *et al*., 2014). The associations between AST, ALT concentration and the incidence of type 2 diabetes have also examined in number of prospective studies (Islam *et al*., 2020). In our study, IL -6 and IL -1β showed significantly no correlation with AST and ALT levels in both healthy and NDD subjects. Our results are also in contrast to previous study that show the significant correlation of pro-inflammatory cytokines with FBS, RBS and HbA1c in T2DM (Sattar *et al*., 2018).

This study indicates the association of IL-6 and IL-1β with related biochemical indexes in NDD subjects in our population is its strength. The study was performed on small sample size is the limitations of this study. It emphasizes the need for further large prospective studies to establish the role of inflammatory biomarkers with various biochemical parameters and its associated chronic diseases in the development of T2DM.

CONCLUSION

With increased IL -6 and IL -1β values in NDD subjects only IL -1β showed significant correlation as compared to IL-6. In addition, significant correlation of IL -1β with various biochemical parameters as compared to IL-6 were also observed to be involved in progression from normoglycemia to type 2 DM.

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