Original Article
Serum cytokine, chemokine and hormone levels in Saudi adults with pre-diabetes: a one-year prospective study

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Abstract: Approximately 5-10% of subjects with pre-diabetes eventually progress to diabetes every year. While inflammation is thought to be involved in the development of obesity-related type 2 diabetes mellitus (T2DM), the relation between inflammation and pre-diabetes remains largely unexplored. In this study we examined a comprehensive panel of 10 serum biomarkers involved in overweight and obese subjects with pre-diabetes. A total of 98 subjects (23 males, 75 females) were advised to reduce total intake of fat, increase fiber intake and physical activity. Serum cytokines, MCP and other hormones were assessed by multiplex cytokine profiling. Results show that CRP, IL-6, leptin, IL-10, MCP, resistin, serpin, and TNF-α were significantly lower after 12-months than baseline. Serum concentrations of other adipocytokines, including adipsin and leptin were modestly lower in the 12-month follow-up than baseline, but failed to reach statistical significance. Changes in HbA1c was found to be positively correlated with adipsin, CRP, IL-6, IL-10, resistin, serpin, and TNF-α. The results suggest that promotion of lifestyle changes for one year among overweight and obese subjects modestly changes several circulating inflammatory biomarkers which maybe favorable in reducing risk for T2DM progression.

Keywords: DMT2, Pre-diabetes, cytokines, inflammation, follow-up

Introduction

Diabetes mellitus is a growing public health problem worldwide [1]. While the prevalence of type 2 diabetes mellitus (T2DM) varies between populations, the prevalence of this disease in Saudi Arabia and the surrounding Middle Eastern Gulf states is among the highest in the world (International Diabetes Federation (IDF); http:\\www.idf.org). According to IDF, Saudi Arabia has the seventh highest prevalence of T2DM in the world and this has doubled over the last two decades, with recent estimate of the disease to be 23.1% [2].

The development of diabetes is an ongoing process beginning with pre-diabetes, a condition characterized by an increased blood glucose concentration that is not high enough to be classified as having diabetes [3]. In 1997, the American Diabetes Association (ADA) defined pre-diabetes as fasting plasma glucose (FPG) 6.1-7.0 mmol/L (110-126 mg/dl) [4]. The choice of the 6.1 mmol/L threshold was based partly on the increased risk of developing both microvascular and macrovascular complications above this level [5]. In 1997, the percentage of Saudis who had an FPG in the impaired fasting glucose range (6.1-7.0 mmol/L) was 14.1% [6]. As such, a better understanding of the pathogenic mechanisms underlying the progression from pre-diabetes to T2DM, is of the utmost clinical importance.

Chronic inflammation plays an important role in the pathophysiology of T2DM [7, 8]. Bertoni et al. reported that higher baseline levels of inter-leukin-6 (IL-6), C-reactive protein (CRP), and fibrinogen were associated with increased inci-dence of T2DM in a multiethnic American cohort [9]. To date; only few studies have exam-ined pro-inflammatory biomarkers in pre-dia-betic individuals. Gupta et al. observed increased levels of interferon (IFN)-C, IL-6, tumor necrosis factor-α (TNF-α), and IL-1β in Irish subjects with pre-diabetes compared to normoglycemic individuals [10]. Higher levels of CRP have been associated with progressively higher risk of impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) in Chinese subjects [11]. Plasma IL-6 was elevated in Italians and Caucasians with IGT and T2DM [12]. Another study reported increased concentrations of TNF-α in Turkish females with IGT than those with normal glucose tolerance (NGT) [13]. In contrast, Choi et al. [14] observed no significant differences in TNF-α and IL-6 concentrations in Korean women with IGT and NGT. Of interest, one study found mark-edly elevated plasma IL-8 concentration after glucose load in obese subjects with IGT as com-pared to NGT [15]. Moreover, hyperglycemia is known to increase the plasma concentrations of IL-6 and TNF-α within few hours and this effect is more distinct in individuals with IGT. However, whether pre-diabetes is character-ized by an increased presence of various pro-inflammatory cytokines, chemokines and hor-mones have not been clearly demonstrated to date, particularly in Saudis, and therefore rep-resent the main aim of this study.

Materials and methods

Study population

A total of 98 (23 males; 75 females) adult Saudi patients with pre-diabetes aged 43.6 ± 10.9 years, were initially recruited to take part in this prospective study. These subjects were recruit-ed as part of the on-going Biomarker Screening Project of King Saud University in collaboration with the Ministry of Health, which began in 2008. Exclusion criteria include: Patients tak-ing mineral oil products, using antacids regu-larly, taking cortisone or other steroids, under diuretics, taking weight-loss drugs, under phe-nobarbital and phenytoin medications, having liver problems, gallbladder disease or gastroin-testinal disorders and taking daily multivita-mins, including calcium. Calcium metabolism abnormalities, such as evidence of metabolic disease (Paget's disease or osteomalacia), hyperparathyroidism, renal stone disease, and abnormal levels of calcium, phosphorous and alkaline phosphatase were also excluded. Ethics approval was granted by the Ethics Committee of the College of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia (KSA). Participating subjects were recruited and enrolled longitudinally in 4 primary health care centers (PHCCs) within the Riyadh Central Region during the summer months (April-July 2009). They were asked to complete a general-ized questionnaire, which contains demograph-ic information, including past and present med-ical history, and to return after overnight fasting for more than 10 hours for anthropometry and blood withdrawal. They were also seen 6 and 12 months later for another anthropometry and metabolic assessments.

Intervention

All subjects were advised and followed-up on their weight reduction of 5% or more, total intake of fat to <30% of energy consumed, intake of saturated fat <10% of energy con-sumed, increased fiber intake to at least 15 g/1000 kcal and moderate exercise for at least 30 minutes per day (30 minute walk, 5 times a week).

Anthropometry and blood collection

Subjects were requested to visit their respec-tive PHCCs in an overnight fasted state (>10 hours) for anthropometry and blood withdrawal by the PHCC nurse and physician on duty, respectively. Anthropometry included height (rounded off to the nearest 0.5 cm), weight (rounded off to the nearest 0.1 kg), waist and hip circumference (centimeters), and mean sys-tolic and diastolic blood pressure (millimeters of Hg) (average of 2 readings). Body mass index was calculated as weight in kilograms divided by height in square meters. Fasting blood sam-ples were collected and transferred immedi-ately to a non-heparinized tube for centrifuga-tion. Collected serum was then transferred to pre-labeled plain tubes; stored in ice; and deliv-ered to the Biomarkers Research Program (BRP) in King Saud University, Riyadh, KSA, for immediate storage at -20°C.

Table 1. Clinical Characteristics at Baseline and 12-months follow up

	Baseline	12 months	*P* value
N		98	
Age (years)		43.6 ± 10.9	
Male/Female		23/75	
Body Mass Index (kg/m^2)	32.7 ± 6.7	32.5 ± 6.5	0.37
Systolic BP (mmHg)	118.2 ± 14.5	116.2 ± 14.6	0.21
Diastolic BP (mmHg)	76.2 ± 9.5	75.7 ± 10.8	0.79
Glucose (mmol/l)	6.5 ± 0.50	5.7 ± 0.86	<0.001
HbA1c (%)	6.1 ± 1.6	5.6 ± 1.3	0.02
Total Cholesterol (mmol/l)	4.6 ± 1.1	4.2 ± 1.0	0.008
Triglycerides (mmol/l)	1.7 ± 0.80	1.5 ± 0.70	0.04
HDL-Cholesterol (mmol/l)	1.1 ± 0.31	0.71 ± 0.35	<0.001
LDL-Cholesterol (mmol/l)	3.5 ± 1.1	3.3 ± 0.97	0.22

Note: Data presented as mean ± standard deviation; *P*-value significant at <0.01.

Table 2. Comparison of measured serum biomarkers between baseline and 12-months follow up

	Baseline	12 months	*P*-value
Adiponectin (μg/ml)	0.82 ± 0.10	1.2 ± 0.15	0.02
Adipsin (μg/ml)	0.31 (0.14, 0.53)	0.21 (0.14, 0.51)	0.16
C-Reactive Protein (μg/ml)	0.04 ± 0.004	0.02 ± 0.001	0.002
IL-6 (pg/ml)	35.8 (21.2, 48.4)	27.1 (20.3, 32.4)	<0.001
Leptin (ng/ml)	18.0 ± 1.3	17.5 ± 1.2	0.75
IL-10 (pg/ml)	28.4 (15.0, 38.9)	21.1 (16.7, 26.3)	<0.001
MCP (pg/ml)	130.8 (90.6, 183.2)	114.8 (83.5, 155.3)	0.03
Resistin (ng/ml)	4.3 ± 0.30	3.5 ± 0.23	0.07
Serpin (ng/ml)	14.3 (7.9, 25.7)	11.4 (7.6, 20.1)	0.04
TNF-α (pg/ml)	91.5 (57.2, 123.3)	79.4 (61.6, 103.5)	0.07

Note: Data represented by Mean ± Std. Dev. Paired sample t-test was done comparing baseline, 12 months. IL-6, IL-10, MCP, Serpin, TNF-α represented by Median Interquartile range; Wilcoxon test done to compare non-Gaussian parameters. Significant at P<0.01.

Sample analyses

Fasting glucose, lipid profile, calcium, and phosphorous were measured using a chemical analyzer (Konelab, Espoo, Finland). All the serum cytokines, chemokines and hormones were measured by Luminex IS 200 (Lincoplex, USA) the protocol was performed as per the manufacturer's instruction (Millipore) with detection across a range of 1-10,000 pg/ml for each analyte.

Data analysis

Data were analyzed using SPSS version 16.0 (SPSS, Chicago, IL, USA). Normal continuous variables were presented as mean ± standard deviation. Paired T-test was done to compare differences between groups and Wilcoxon Rank test for Non-Gaussian variables. Correlation analyses were performed using delta serum HBA1c as dependent variable. Significance was set at P<0.05 as marginally significant and <0.01 as significant.

Results

Clinical characteristics of study subjects in baseline and 12-month groups

Data from the 98 patients were analyzed and reported as mean (SD) as demonstrated in **Table 1**. The mean age of the patients was 43.6 ± 10.9 years; 75% were women, and 25.0% men. There were no significant differences in BMI, systolic BP, and diastolic BP from baseline in any of the groups up to 12 months. However, improvements were observed in the lipid profile after 12-months compared to baseline. The mean glucose, HbA1C, triglycerides, total and HDL cholesterol were modestly lower in the 12-month follow-up than baseline (*P*<0.05).

Comparison of serum biomarkers between both groups

We compared serum levels of a panel of biomarkers associated with inflammation, including adiponectin, adipsin, CRP, IL-6, leptin, IL-10, MCP, resistin, serpin, TNF-α between baseline group and their matched 12-month follow-up group. As shown in **Table 2**, serum concentrations of CRP, IL-6, leptin, IL-10, MCP, resistin, serpin, and TNF-α were significantly lower in 12-month follow-up compared to baseline. Adiponectin was modestly higher after 12 months compared to baseline (*P*=0.02). Serum concentrations of several adipocytokines,

Table 3. Correlations of HbA1c with measured serum biomarkers in the entire cohort

	Δ HbA1c	
	r	*P* value
Δ Adiponectin (μg/ml)	-0.10	0.34
Δ Adipsin (μg/ml)	0.22	0.04
Δ CRP (μg/ml)	0.40	<0.001
Δ IL-6 (pg/ml)	0.24	0.03
Δ Leptin (ng/ml)	0.16	0.13
Δ IL-10 (pg/ml)	0.29	0.005
Δ MCP (pg/ml)	0.10	0.34
Δ Resistin (ng/ml)	0.22	0.04
Δ Serpin (ng/ml)	0.22	0.04
Δ TNF-α (pg/ml)	0.21	0.04

r-Pearson's bivariate correlation coefficient. Δ-Delta values were used. Note: Significant at *P*<0.01.

including adipsin and leptin were lower in the 12-month follow-up than baseline (**Table 2**), but failed to reach statistical significance.

Associations of HbA1c with serum biomarkers in the overall population

Prospective changes in the measured biomarkers in all subjects were evaluated for its associations with changes in HbA1c. Adipsin, resistin, serpin and TNF-α had borderline positive associations with HbA1c (*P*-values 0.04, respectively) while CRP (R=0.40; *P*<0.001) and IL-10 (R=0.29; *P*=0.005) had stronger positive associations with HbA1c. The rest of the biomarkers measured did not correlate with HbA1c (**Table 3**).

Incidence of T2DM

From baseline, about 68% became normoglycemic, 20% still had pre-diabetes and 11% progressed to T2DM after 12-months.

Discussion

To the best of our knowledge, this is the first study to prospectively evaluate the various serum cytokines, chemokines and hormones in Saudi subjects with pre-diabetes. Among the most interesting results, 60% of patients with pre-diabetes at baseline lowered their glucose levels to normal status after 12 months. The baseline group displayed a pro-inflammatory state with higher serum levels of CRP, IL-6, leptin, IL-10, MCP, resistin, serpin, and TNF-α.

These findings suggest that subclinical inflammation is already in progress as early as pre-diabetes and that inflammation is a major component of pre-diabetes. This observation highlights the importance of early intervention in the pre-diabetes state to prevent progression to T2DM and reduce CVD risk.

Recent studies have demonstrated that inflammation is involved in the development of T2DM [16, 17]. T2DM is now recognized as an immune-mediated disease leading to impaired insulin signaling and selective destruction of insulin producing β-cells, in which cytokines play an important role [18]. TNF-α and IL-6 were previously observed to be involved in the development of T2DM [18, 19]. Our results support these previous reports of higher levels of TNF-α and IL-6 in individuals with impaired glucose tolerance IGT [17], but contradict the findings of Choi et al. [14] who observed no differences in TNF-α and IL-6 concentrations between Korean women with IGT. The discrepancy in the findings could be due to variations in sample size, BMI status, race, geographical conditions, and the definition of pre-diabetes, since we used IGT, rather than HbA1c criteria, to define pre-diabetes.

The observation of increased CRP levels among subjects at baseline was rather interesting. Previous reports suggested that obesity may play an important role in the relation between CRP and pre-diabetes. Current evidence suggests obesity induces low-degree chronic inflammation through enhanced adipose tissue-derived cytokine expression (e.g., IL-6, and TNF-α). These pro-inflammatory cytokines regulate and promote CRP synthesis. The association between CRP and pre-diabetes is largely consistent with previous studies that report an association between CRP and pre-diabetes elevated blood glucose levels and HbA1c [21]. Adiponectin, which was elevated in our subjects after 12-months compared to baseline, was observed to be lower in men and women with metabolic syndrome and T2DM. Adiponectin, a possible mediator of triglyceride accumulation, appears to have an important role not only in insulin resistance but in obesity-related cancers as well [22].

Another interesting observation in our study was the higher levels of IL-10 at baseline than after 12-months follow up. IL-10 plays a central

role in regulating immune response and limiting inflammation. It suppresses inflammation through suppression of p65 NF-kB and creative activity in macrophages [20]. IL-10 also down regulates the release of reactive oxygen species and nitrogen intermediates antigen presentation capacity and suppression of proliferative and cytotoxic T cell responses [21]. There is growing evidence linking IL10 to obesity, [22] metabolic syndrome (MetS) and cardiovascular disease. In adults, low circulating IL10 has been associated with obesity [22], cardiovascular disease [23] and T2DM [23]. Clinical significance of circulating IL10 concentrations have been demonstrated in acute coronary syndrome and insulin resistance [23].

Circulating MCP-1 and resistin were found to be modestly higher at baseline. It was reported that MCP-1 is stimulated by chronic hyperglycemia. MCP-1 is a C-C chemokine that exhibits its most potent chemotactic activity toward monocytes and T-cells [24]. In addition to promoting the transmigration of circulating monocytes into tissues, MCP-1 exerts various effects on monocytes, including the induction of superoxide anion [25]. Cytokine production, and adhesion molecule expression. The present data and other studies suggest that MCP-1 may alter adipocyte function and metabolism which may contribute to the development of insulin resistance and adipocyte differentiation. Resistin plays a role in glucose homeostasis both in mice and humans, and was recently observed to be elevated among smokers with T2DM [26].

There is scarcity of evidence on the association between HbA1c and the different metabolic analytes measured in the study. One study found positive correlations of HbA1c with CRP, fibrinogen, albumin, erythrocyte sedimentation rate, and white blood cell counts in older patients with coronary artery disease [27]. However, Martins et al. saw no correlation of HbA1c with CRP in older adults [28]. Although the mechanisms underlying the relation between inflammation, obesity, and impaired glucose homeostasis or elevated HbA1c are still incompletely understood, certain pro-inflammatory cytokines have been recognized as central players [29]. TNF-α in the adipose tissue is associated with an increased recruitment of activated macrophages [30]. While the IjB kinase-b (IKKb) pathway is identified as a

target for TNF-α-induced insulin resistance [31], TNF-α down-regulates the tyrosine kinase activity of the insulin receptor and has also been implicated in the induction of cardiac insulin resistance [32]. On the other hand, IL-6 is involved in the regulation of the acute phase response and insulin resistance [29]. Subcutaneous and visceral fat has been demonstrated as an important site for IL-6 secretion in humans.

In our study, adipsin, resistin and serpin were positively correlated with HbA1c, as did CRP, IL-6, IL-10, MCP and TNF-α. In addition, the degrees of correlations among these biomarkers could be due to numerous pathophysiological factors such as their roles in the inflammation pathway, their interactions in response to inflammatory stimuli, various related transcriptional factors, and the balance between Th1 and Th2 phenotypes. How their correlations are varied and involved in obesity and glucose homeostasis deserves further investigation in cells, animal models, and human populations.

One possible limitation of this study is that the pathway from pre-diabetes to diabetes could not be adequately modeled using our data. In addition, because we studied only Saudis, our results may not apply to other ethnicities. Several confounding founders that were not included in the present study include cardiovascular medications which can alter the cytokine profile of subjects as well as lifestyle habits including, physical activity, sleep quality and duration which are also known to affect inflammatory markers [33]. Nevertheless, there are only a few studies analyzing several biomarkers in pre-diabetes, whereas the present study included a panel of 10 cytokines, chemokines and hormones to define pre-diabetes. Our data highlights the involvement of less commonly studied biomarkers including adipsin, resistin, serpin, IL-10 and MCP which appeared to modestly change after one year of lifestyle modification.

In conclusion, the present observations highlight modest but favourable changes in the pro-inflammatory cytokines, chemokines and hormones among pre-diabetes patients who underwent one year lifestyle modifications. Whether these favourable changes decrease or delay subsequent progression to T2DM requires longer investigation.

Disclosure of conflict of interest

None.

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