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Inflammatory cytokines and immune system response to weight reduction in obese patients with type 2 diabetes mellitus

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ABSTRACT

Background: Systemic inflammation and activated immune system response are common features in obese patients with non-insulin dependent diabetes mellitus (NIDDM) as obesity-induced NIDDM represents a burden for healthcare systems worldwide. However, there is a strong association between BMI and the human immune system and systemic inflammation among obese patients with NIDDM.

Objective: This study aimed to examine effects of weight reducing program on selected immune and systemic inflammation parameters among obese patients with NIDDM.

Material and Methods: Eighty obese patients with NIDDM participated in this study, their age ranged from 41-52 years and their BMI ranged from 31-36 kg/m^2. All Subjects were included in two groups: The first group received life style modification in the form of treadmill aerobic exercises in addition to diet control where, the second group received no therapeutic intervention. Parameters of CD4 and CD8 cells count were quantified, IL-6, TNF-α, leptin and body mass index (BMI) were measured before and after 3 months at the end of the study.

Results: The mean values of CD4 and CD8 cells count were significantly increased, where mean values of TNF-α, IL-6, IL-8 and body mass index (BMI) were significantly decreased in group (A). While group (B) showed non-significant changes in these parameters. Also; there were significant differences between mean levels of the investigated parameters in group (A) and group (B) at the end of the study.

Conclusion: Within the limit of this study, life style modification modulates systemic inflammation and immunological parameters among obese patients with NIDDM.

Keywords: obesity, type 2 diabetes mellitus, immune system, cytokines, weight reduction

INTRODUCTION

Non-insulin dependent diabetes mellitus (NIDDM) is now a worldwide epidemic (1) and more than 350 million people will have diabetes by 2030 and it is strongly correlated with an elevated incidence of obesity (2).

A chronic low-grade inflammation and an activation of the immune system are observed in abdominal obesity and may have a role in the pathogenesis of obesity-related metabolic disorders (3, 4). Inflammatory responses may have a dual role in NIDDM, since it may have either a causal relationship leading to resistance to insulin or may be intensified by the hyperglycemic state, resulting in NIDDM complications (5).

Obesity has also been associated with decreased immunocompetence as it alters innate and adaptive immunity and immunity deterioration is related to the grade of obesity (6). Also, there is an association of obesity with 25–40% of certain malignancies in both obese men and women (7). Many authors reported dysregulation and alteration in number of immune cells in obese subjects as elevated numbers of circulating immune cells as neutrophil, monocyte, leukocyte and total white blood cells (8-10). In addition, body mass index is positively correlated with the number of macrophages in adipose tissue (11).

As there is limitation in studies reporting the benefits of lifestyle modification on immune system response among obese type 2 diabetic patients. This study aimed to examine effects of weight reducing program on selected immune and systemic inflammation parameters among obese patients with NIDDM.

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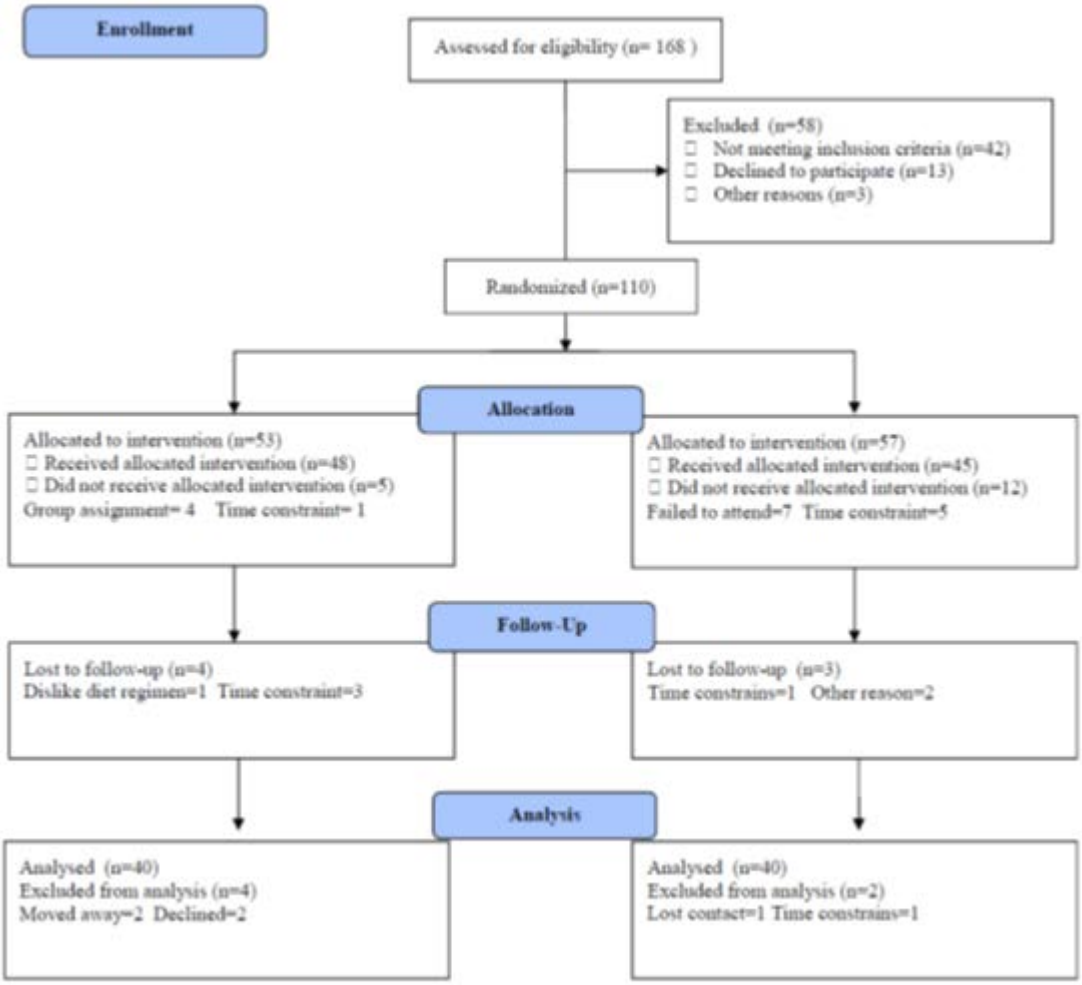

Figure 1: *Subjects screening and recruitment CONSORT diagram*

PATIENTS AND METHODS

Subjects

Eighty obese patients with NIDDM; their age ranged from 41 to 52 years, treated with oral hypoglycemic agents e.g. metformin and/or pioglitazone were selected studied on referral to Internal Medicine Department, King Abdulaziz University Teaching Hospital, Saudi Arabia. Exclusion criteria included patients with renal, cardiac and liver diseases. All participants will be free to withdraw from the study at any time. Following pre-training testing, all participants were enrolled into two equal groups: group (A): received weight reduction program in the form of treadmill aerobic exercises in addition to diet control, where group (B): received no therapeutic intervention. The CONSORT diagram outlining the details of the screening, run-in and randomization phases of the study and reasons for participant exclusion can be found in **Figure 1**. Informed consent was obtained from all participants. This study was approved by the Scientific Research Ethical Committee, Faculty of Applied Medical Sciences at King Abdulaziz University.

Measurements

The following measurements were taken before the study and after 3 months at the end of the study.

A. **Inflammatory cytokines:** Venous blood samples were collected in polystyrene tubes after a 12-h fasting, by venipuncture of the antecubital vein while patients rested in a supine position. The blood samples were transported to a laboratory within 1 h and centrifuged at + 4 °C to remove serum (1000 = g for 10 min). Serum IL-6 levels were measured using "Immulite 2000" immunassay analyzer (Siemens Healthcare Diagnostics, Deerfield, USA). However, interleukin-8 (IL-8) and TNF-α levels were analyzed with ELISA kits using ELISA microplate strip washer (ELX 50), and ELISA microplate reader (ELX 808; BioTek Instruments, USA).
B. **Flow cytometry analysis:** The human leukocyte differentiation antigens CD4 and CD8 (Beckman Coulter, Marseille, France) Five microliters of appropriate monoclonal antibody was added to 50 µL of a whole-blood

Table 1: *Baseline characteristics of study participants*

Characteristic	Group (A)	Group (B)	Significance
Age (years)	46.71 ± 5.26	47.35± 6.13	P > 0.05
BMI (kg/m^2)	32.43 ± 3.82	31.56 ± 4.11	P > 0.05
SBP (mm Hg)	143.26 ± 8.91	144.16 ± 7.35	P > 0.05
DBP (mm Hg)	86.27 ± 5.31	84.98 ± 6.58	P > 0.05
Fasting glucose (mg/dl)	125.12 ± 7.36	123.73 ± 6.52	P > 0.05
HbA1c (%)	7.35 ± 1.21	7.22 ± 1.43	P > 0.05
Total cholesterol (mg/dl)	190.74 ± 10.33	193.97 ± 9.66	P > 0.05
HDL-cholesterol (mg/dl)	32.61± 2.48	31.15 ± 2.62	P > 0.05
LDL-cholesterol (mg/dl)	134.14 ± 8.22	136.13 ± 8.16	P > 0.05
Triglyceride (mg/dl)	155.88 ± 11.34	158.17± 10.32	P > 0.05

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HBA1c: Glycosylated hemoglobin; HDL= High Density Lipoprotein; LDL= Low Density Lipoprotein

sample and incubated for 15 minutes at room temperature. Thereafter, the erythrocytes were lysed with 125 µL of a lysing solution, OptiLyse C, for 10 minutes. The reaction was stopped by the addition of 250 µL phosphate-buffered saline. The samples were analyzed by flow cytometry using Cytomics FC 500 and CXP software (Beckman Coulter).

C. **Body mass index (BMI):** The participants were measured whilst wearing their undergarments and hospital gowns. Height was measured with a digital stadiometer to the nearest 0.1 cm (JENIX DS 102, Dongsang, South Korea). Body weight was measured on a calibrated balance scale to the nearest 0.1 kg (HC4211, Cas Korea, South Korea), and BMI was calculated as BMI = Body weight / (Height)^2.

Procedures

Following the previous evaluation, all patients will be divided into the following groups:

1. **The training group (Group A)** received aerobic exercise training for 3 months on the treadmill (Enraf Nonium, Model display panel Standard, NR 1475.801, Holand) which was conducted according to recommendation of aerobic exercise application approved by the American College of Sports Medicine (12). Training program included 5 minutes for warming–up in the form of range motion and stretching exercises , 30 minutes of aerobic exercise training with intensity equal 60-70% of the individual maximum heart rate followed by cooling down for 10 minutes (on treadmill with low speed and without inclination). Participants had 3 sessions /week for 3 months with close supervision of physical therapist. Also, a dietician performed an interview-based food survey for all participants of group (A) for detection of feeding habits, abnormal dietary behavior and to prescribe the balanced low caloric diet (13) that provided 1200 Kilocalories/day for 3 months. The same dietitian continuously monitored all participant caloric intakes through reviewing the detailed record of food intake every 2 weeks (14, 15).
2. **The control group (Group B)** received no exercise intervention or diet regimen.
3. **Statistical Analysis:** The mean values of the investigated parameters obtained before and after three months in both groups were compared using paired "t" test. Independent "t" test was used for the comparison between the two groups ($P<0.05$).
4. **Results:** Eighty obese patients with NIDDM completed the screening evaluation, none of the baseline characteristics differed significantly between the two groups as listed in **Table 1**.
   In the lifestyle intervention group of NIDDM patients, the mean values of BMI, TNF-α, IL6 and IL8 were considerably reduced to significant levels, while the mean values of CD4 cell count and CD8 cell count were considerably increased to significant levels over the period of therapy (**Table 2**). In the other hand, the results of the control group were not significant (**Table 3**). Also, there were significant differences between both groups at the end of the study (**Table 4**).

Table 2: *Mean value and significance of TNF-α, IL-6,IL-8, BMI, CD4 cell count and CD8 cell count in group (A) before and at the end of the study*

	Mean + SD		t- value	Significance
	Pre	Post		
BMI (kg/m^2)	32.43 ± 3.82	28.24 ± 2.76*	5.28	P <0.05
TNF- α (pg/mL)	13.16 ± 2.87	9.13 ± 2.45*	6.11	P <0.05
IL-6 (pg/mL)	5.49 ± 1.25	3.61 ± 1.28*	6.54	P <0.05
IL-8 (pg/mL)	17.12 ± 3.24	14.22 ± 3.19*	5.52	P <0.05
CD4 count (10^9/L)	1.21 ± 0.66	1.56 ± 0.78*	5.75	P <0.05
CD8 count (10^9/L)	0.57 ± 0.27	0.88 ± 0.36*	5.14	P <0.05

BMI: Body Mass Index; TNF-α: tumor necrosis factor – alpha; IL-6: Interleukin-6; IL-8: Interleukin-8; (*) indicates a significant difference between the two groups, P < 0.05.

Table 3: *Mean value and significance of TNF-α, IL-6,IL-8, BMI, CD4 cell count and CD8 cell count in group (B) before and at the end of the study*

	Mean + SD		t- value	Significance
	Pre	Post		
BMI (kg/m^2)	31.56 ± 4.11	31.78 ± 4.15	0.87	P>0.05
TNF- α (pg/mL)	12.52 ± 3.18	12.77 ± 3.14	0.96	P>0.05
IL-6 (pg/mL)	5.59 ± 1.51	5.72 ± 1.58	1.23	P>0.05
IL-8 (pg/mL)	17.66 ± 3.34	18.01 ± 3.41	1.47	P>0.05
CD4 count (10^9/L)	1.18 ± 0.71	1.13 ± 0.69	0.82	P>0.05
CD8 count (10^9/L)	0.55 ± 0.26	0.54 ± 0.27	0.73	P>0.05

BMI: Body Mass Index; TNF-α: tumor necrosis factor – alpha; IL-6: Interleukin-6; IL-8: Interleukin-8; (*) indicates a significant difference between the two groups, P < 0.05

Table 4: *Mean value and significance of TNF-α, IL-6,IL-8, BMI, CD4 cell count and CD8 cell count in group (A) and group (B) at the end of the study*

	Mean + SD		t- value	Significance
	Group (A)	Group (B)		
BMI (kg/m^2)	28.24 ± 2.76*	31.78 ± 4.15	5.71	P <0.05
TNF- α (pg/mL)	9.13 ± 2.45*	12.77 ± 3.14	6.26	P <0.05
IL-6 (pg/mL)	3.61 ± 1.28*	5.72 ± 1.58	6.82	P <0.05
IL-8 (pg/mL)	14.22 ± 3.19*	18.01 ± 3.41	5.63	P <0.05
CD4 count (10^9/L)	1.56 ± 0.78*	1.13 ± 0.69	5.85	P <0.05
CD8 count (10^9/L)	0.88 ± 0.36*	0.54 ± 0.27	5.70	P <0.05

BMI: Body Mass Index; TNF-α: tumor necrosis factor – alpha; IL-6: Interleukin-6; IL-8: Interleukin-8; (*) indicates a significant difference between the two groups, P < 0.05

DISCUSSION

There is a growing concern for NIDDM as the next big therapeutic challenge because of the possible evolution of NIDDM toward different medical complications. The novel of this study is that although exercise and diet improvement may reduce the overall magnanimity of insulin resistance, hyperlipidemia and abnormal cytokine metabolism, there has been only limited research on the effects of weight reduction as the sole intervention on these abnormal biochemical parameters in individuals with NIDDM. However, the limitation of this study is no recoding of the histological changes to the treatment intervention. The aim of this study was to detect changes in immune and systemic inflammation parameters following weight loss in obese patients with NIDDM. The mean values of TNF-α, IL-6, IL-8 and BMI were significantly decreased in group (A), where the mean value of CD-4 cell count and CD-8 cell count were significantly increased, while there were no significant changes in group (B). Also; there was a significant difference between both groups at the end of the study, these findings are supported and agreed by several previous studies (18-25).

Results of our study was confirmed with Dandona et al. Who reported that weight loss reduces TNF-α in obese (16). Also, Sandoval and Davis approved that patients who had bariatric surgery gained reduction in IL-6 concentration and improved insulin sensitivity in parallel to weight loss (17). However, Loria-Kohen and colleagues conducted a study of combining strength or endurance training with a hypocaloric diet for 22 weeks, all groups showed a significantly reduced energy intake (P < 0.001) and improved anthropometric variables (P < 0.001). Tumor necrosis factor-a (TNF-α), and C-reactive protein (CRP) concentrations decreased in all groups when examined together, but not when examined separately. No significant differences were seen in interleukin-6 (IL-6) (18). Also, Balagopal et al. reported that obese adolescents who underwent a 3-month lifestyle intervention of enhanced physical activity and nutrition habits had decreased body fat percentage, insulin resistance and IL-6 (19). Likewise, an exercise intervention of 3 years, which gave

detailed advice in regard to physical activity, in 60 obese women, resulted in weight loss along with decreased levels of TNF-α (20). Moreover, You and Nicklas & Nicklas and colleagues stated that weight loss leads to reductions in circulating IL-6, TNF-α and CRP levels regardless of the way in which the weight loss was achieved, including hypocaloric dietary intake, exercise, or liposuction (21, 22). The three possible mechanisms of exercise anti-inflammatory effects include reduction in visceral fat mass (23); reduction in the circulating numbers of pro-inflammatory monocytes (24) and an increase in the circulating numbers of regulatory T cells (25).

A major finding of this work is that weight loss after caloric restriction and exercise training may restore immune function. The results of our study agree with several previous studies suggesting improvements in body composition promote the modulation of immune system markers (26-29), where Wasinski and colleagues founded that weight reduction in mice submitted to a high-fat diet resulted in macrophage reduction promoted by exercise and caloric restriction was followed by an increased number of CD8+ T and CD4+ T cells. The reduction of these cells induced by exercise and caloric restriction suggests that inflammation improvement in adipose tissue induces a reduction in CD4+ T cells (26). Also, Lamas and colleagues stated that a 4-week energy restriction (50% of total energy intake) is able to restore, at least in part, the impaired immune response in previously diet-induced (cafeteria) overweight rats (27). The possible mechanisms of exercise immune system modulating effects include decreased levels of proinflammatory cytokines TNF-α (28), IL-6 (29) and C-reactive protein (30) along with an increase in the interleukin -10 (IL-10) (29).

The current study has important strengths and limitations. The major strength is the supervised nature of the study. Supervising food intake and physical activity removes the need to question compliance or to rely on food and activity questionnaires. Further, all exercise sessions were supervised and adherence to the diet and activities was essentially 100%. Moreover, the study was randomized; hence, we can extrapolate adherence to the general population. In the other hand, the major limitations is the small sample size in both groups may limit the possibility of generalization of the findings in the present study. Finally, within the limit of this study, life style modification modulates systemic inflammation and immunological parameters among obese patients with NIDDM.

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CONCLUSION

Within the limit of this study, life style modification modulates systemic inflammation and immunological parameters among obese patients with NIDDM.

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