INTRODUCTION

Inflammatory mechanism is believed to take part in the pathogenesis of diabetes and at risk individuals for T2DM demonstrated features of low grade inflammation prior to the onset of the disease. Low grade inflammation is considered as contributory factor for the pathogenesis of T2DM. Elevated level of systemic inflammatory markers IL-6 and C-reactive protein (CRP) predicts the development of diabetes. Therefore, increased level of IL-6 and CRP is considered an independent risk for the onset of diabetes and supported the possible role of inflammation in the pathogenesis of T2DM. Laboratory investigation of IL-6 and CRP could be an adjunct method for the early diagnosis of T2DM. Hence, there is association between increased circulatory level of IL-6 and the onset of T2DM. Nitric oxide synthase is an enzyme which promotes the production of nitric oxide, which in turn has a key function in insulin secretion along with angiogenesis, neural development, vascular and airway tone. Literature also supports the role of nitric oxide synthase in the development of neuropathies in patients with diabetes. Nitric oxide synthase has also a major role in the control of arterial pressure with glucose and lipids homeostasis. It also contributes to the pathogenesis of renal hemodynamic changes in DM and appears to be a key isoform in the production of hemodynamically dynamic NO in this condition. Cyclooxygenase mediated inflammation is associated with the development of T2DM in elderly population by increase in prostaglandin formation. Increased level of cytokine-mediated acute phase proteins in male population with diabetes has association with obesity.

ORIGINAL ARTICLE

Effects of Supervised Structured Aerobic Exercise Training Program on Interleukin-6, Nitric Oxide Synthase-1, and Cyclooxygenase-2 in Type 2 Diabetes Mellitus

Hossein Karimi1, Syed Shakil-ur-Rehman1,2 and Syed Amir Gillani1

ABSTRACT

Objective: To determine the effects of supervised structured aerobic exercise training (SSAET) program on interleukin-6 (IL-6), nitric oxide synthase 1 (NOS-1), and cyclooxygenase-2 (COX-2) in type 2 diabetes mellitus (T2DM).

Study Design: Randomized controlled trial.

Place and Duration of Study: Riphah Rehabilitation and Research Centre, Railways General Hospital, Rawalpindi, from January 2015 to June 2016.

Methodology: Patients of either gender of minimum one year history of T2DM ranging from 40-70 years of age were included. Those with chronic systemic diseases, history of regular exercise, smoking, and those on dietary plan were excluded. A total of 195 patients were screened; 120 were selected and 102 agreed to participate in the study. They were randomly placed into experimental and control groups. SSAET program, routine medication, and dietary plan were applied in experimental group; whereas, control group was managed with routine medication and dietary plan for 25 weeks. IL-6, NOS-1, and COX-2 were assessed at baseline and 25 weeks.

Results: SSAET program, routine medication and dietary plan showed significantly improved IL-6 (pre-mean=0.25 ±0.11 ng/ml, post-mean=0.19 ±0.04 ng/ml), NOS-1 (pre-median=4.65 ng/ml, IQ range=1.04 ng/ml), (post-median=2.72 ng/ml, IQ range=1.60 ng/ml), and COX-2 (pre-mean=18.72 ±4.42 ng/ml, post-mean=15.18 ±2.63 ng/ml) in experimental group, as compared with control group managed by routine medication and dietary plan; where deterioration was noted in IL-6 (pre-mean=0.23 ±0.08 ng/ml, post-mean=0.27 ±0.08 ng/ml) and COX-2 (pre-mean=18.49 ±5.66 ng/ml, post-mean=19.10 ±4.76 ng/ml), while NOS-1 slight improvement (pre-mean=4.99 ng/ml, IQ range=2.67 ng/ml), (post-mean=4.56 ng/ml, IQ range=3.85 ng/ml). Statistically at the baseline the p-values were not significant (p>0.05) in both experimental and control groups for IL-6, COX-2 and NOS-1; while after 25 weeks of intervention, the experimental group showed significant improvement (p<0.05) in comparison with the control group.

Conclusion: SSAET program, routine medication, and dietary plan had positive effect on IL-6, NOS-1, and COX-2 in T2DM patients.

Effects of supervised structured aerobic exercise training program on interleukin-6, nitric oxide synthase-1, and cyclooxygenase-2 in type 2 diabetes mellitus

and increased fasting insulin. Chronic inflammation is suggested as early sign of pathogenesis of diabetes, while later on inflammation causes oxidative injury in patients with diabetes. Additionally, activation of COX-2 pathways is a key mediator in neurochemical and neurovascular defects in experimental diabetes mellitus (EDM). Glucose-mediated oxidative stress and changes in COX pathway activity with secondary deficits of endoneurial perfusion have implications in the EDM-related neuropathy. Literature reported the vital role of COX-2 and its pathways in the pathogenesis and complications of T2DM.

Physical activity and exercise significantly reduce the level of inflammatory biomarkers in metabolic syndrome associated with T2DM irrespective of weight loss. Six months of aerobic type of exercise without significant weight reduction positively affects metabolic status and produce anti-inflammatory effects on individuals with T2DM, as compared with non-exercise control group. IL-6 and other cytokines present in muscles cells are also known as myokines. Physical activity and exercise have positively targeted these myokines and provide protection against low grade inflammation associated with chronic diseases like T2DM and cardiovascular diseases. The current study was designed to determine the effects of supervised structured aerobic exercise training program on IL-6, NOS-1, and COX-2 in T2DM.

METHODOLOGY

This randomized controlled trial was carried out at Riphah Rehabilitation and Research Centre (RRRC), Pakistan Railways General Hospital, Rawalpindi, Pakistan, from January 2015 to June 2016. Inclusion criteria; patients of either gender with T2DM aged 40 to 70 years and minimum one year history after diagnosis on WHO criteria; whereas patients with the history of chronic systemic diseases, smoking, regular exercise and diet plan were excluded.

A pilot study was conducted on 20 patients and sample size was initially calculated by Epitools online calculator. Statistical parameters used were mean of population 1 (0.4340), mean of population 2 (0.6402), variance (0.137), confidence level (0.95), and power (0.8). Sample size per group was 51, and total sample size was 102. A comprehensive screening program was conducted as per inclusion criteria on 195 patients with T2DM. Of them, 120 fulfilled the criteria, and 102 finally agreed to participate. All the study participants were randomly placed in experimental (n=51) and control (n=51) groups.

SSAET combined with routine medication and dietary plan was applied to experimental group, while control group was treated with routine medication and dietary plan for three days in a week for 25 weeks. Blood samples of 5 ml was taken at baseline and on the completion of 25 weeks intervention plan in yellow tube, centrifuged for the period of 10 minutes and serum was separated for the laboratory investigation IL-6, NOS-1, and COX-2.

AssayMax Human IL-6 ELISA kit, made by Assaypro Co, USA, was used for the estimation of IL-6. Estimation of NOS-1 was done by human NOS-1 ELISA kit of Elabscience, China. Human Prostaglandin-2 ELISA kit was used for the quantitative estimation of COX-2. Postgraduate research laboratory of Islamic International Medical College (IIMC) was used for the laboratory work and storage of the blood samples. Written permission was taken from Institutional Review Board (IRB) of the University of Lahore and Ethical Review Committee (IRC) of Riphah College of Rehabilitation Sciences, Riphah International University, and all study participants.

SSAET program was applied by medically graded treadmill, along with telemetric supervision of heart rate, blood pressure, oxygen saturation and ECG. SSAET program of 25 weeks was divided into five phases of five weeks each. In phase-1, the duration of single session was 10 minutes and the total duration per week was 30 minutes; and 30 minutes per week increase was followed in the subsequent four phases. Normal speed of each participant was calculated by 20 meter distance test and considered as treadmill speed during the whole intervention program. Inclination of the treadmill was zero degree with the ground in phase-1, 3 degree in phase 2, and 3 degree increase was followed in the subsequent 3 phases. SSAET program was applied along with routine medication and diet plan in experimental group, while intervention in control group was routine medication and diet plan.

Statistical analysis was done on SPSS software version-20. Shapiro-wilk test was applied to estimate the normality at baseline on both experimental and control groups. IL-6 and COX-2 were find normality distributed and independent t-test were applied while the data for NOS-1 was not normally distributed and Mann Whitney. Statistical tests were applied at 95% (P<0.05) level of significance.

Descriptive statistics of the study sample was assessed and documented. Mean age of the 102 participants was 54 ±8.17 years with mean age of 53.74 ±8.75 years in experimental group and 55.08 ±7.67 years in control group. The gender distribution of the sample was 66.7% (n=68) women and 33.3% (n=34) men.

RESULTS

Ninety-nine percent (97%) study participants were married and only 2.94% (n=3) were single. Mean of years with T2DM after diagnosis was 7.12 ±4.50 years ranging from 1-16 years, and only 18.62 % (n=19) were with past history of smoking.

Clinically, the SSAET program, routine medication and dietary plan more significantly improved IL-6 (pre-mean=0.25 ±0.11 ng/ml, post-mean=0.19 ±0.04 ng/ml),
Table I: Showing comparison of mean, standard deviation and p-value in experimental and control groups for interleukin-6 (IL-6) and cyclooxygenase-2 (COX-2) in type 2 diabetes mellitus.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre mean ±SD Exp group (ng/ml)</th>
<th>Pre mean ±SD Control group (ng/ml)</th>
<th>p-value</th>
<th>Post mean ±SD Exp group (ng/ml)</th>
<th>Post mean ±SD Control group (ng/ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-6</td>
<td>0.25 ±0.11</td>
<td>0.23 ±0.08</td>
<td>0.259</td>
<td>0.19 ±0.04</td>
<td>0.27 ±0.08</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Cyclooxygenase-2</td>
<td>18.72 ±4.42</td>
<td>18.49 ±4.56</td>
<td>0.799</td>
<td>15.18 ±2.63</td>
<td>19.10 ±4.76</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

Table II: Showing comparison of median, Z-value and P-value in experimental and control groups for nitric oxide synthase 1 (NOS-1).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Median (IQ range) in experimental group (ng/ml)</th>
<th>Median (IQ range) in control group (ng/ml)</th>
<th>Z-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-nitric oxide synthase 1</td>
<td>4.85 (1.04)</td>
<td>4.99 (2.67)</td>
<td>-5.12</td>
<td>0.609</td>
</tr>
<tr>
<td>Post-nitric oxide synthase 1</td>
<td>2.72 (1.60)</td>
<td>4.56 (3.85)</td>
<td>-3.33</td>
<td>0.001</td>
</tr>
</tbody>
</table>

NOS-1 (pre-median=4.65 ng/ml, IQ range=1.04 ng/ml, post-median=2.72 ng/ml, IQ range=1.60 ng/ml), and COX-2 (pre-mean=18.72 ±4.42 ng/ml, post-mean=15.18 ±2.63 ng/ml) in experimental group, as compared with control group managed by routine medication and dietary plan, whereas deterioration was noted in IL-6 (pre-mean=0.23 ±0.08 ng/ml, post-mean=0.27 ±0.08 ng/ml) and COX-2 (pre-mean=18.49 ±4.56 ng/ml, post-mean=19.10 ±4.76 ng/ml), while NOS-1 slight improvement (pre-median=4.99 ng/ml, IQ range=2.67 ng/ml, post-median=4.56 ng/ml, IQ range=3.85 ng/ml).

Statistically, at the baseline the p-values were not significant (p>0.05) in both experimental and control groups for IL-6, COX-2 and NOS-1; while after 25 weeks of intervention, the experimental group showed significant improvement (p-value<0.05) in comparison with the control group. Further detailed comparison is given in Tables I and II.

DISCUSSION

Results of the current study showed that patients in experimental group treated with SSAET program, routine medication, and dietary plan had significant improvement in IL-6, NOS-1, and COX-2 in comparison with the patients in control group treated with routine medication, and dietary plan, where IL-6 and COX-2 deteriorated and NOS-1 slightly improved.

Colbert and team conducted a cross-sectional study and investigated the association between physical activity and inflammatory markers with consideration for body fatness and antioxidant use. They concluded that higher level of exercise is associated with lower level of IL-6 and other inflammatory markers among older adults who use antioxidant supplement while compared with non-exercised group. This change was noted regardless of exercise level. The percent study also demonstrated significant improvements in IL-6 in exercise group treated with SSAET program, routine medication and dietary plan.

Jorge and colleagues conducted an experimental study and investigated the effects of resistance, aerobic and combined exercise on inflammatory markers in patients with T2DM. The results demonstrated favorable improvement of 12 weeks exercise along with other benefits of increased glycemic control, improved lipid profile and blood pressure. Intervention was divided into four groups of aerobic, resistance, combined, and non-exercise group. All the three groups aerobic, resistance and combined showed improvements in inflammatory markers, glycemic control, blood pressure and lipid profile more significantly than the non-exercised control group. The present study results showed marked improvement in IL-6, NOS-1, and COX-2 among group of patients treated with SSAET program, routine medication and dietary plan.

Mathur and Pedersen concluded in a review that chronic non-communicable diseases (CNCDs) are getting to epidemic proportions globally, including cancers, heart diseases and T2DM, while it is now clear that a low grade inflammation plays vital role in the pathogenesis of CNCDs. Exercise is suggested for providing protection against CNCDs by controlling low grade inflammation and improving IL-6 like myokines. The current study also concluded that SSAET program, routine medication and dietary plan produced significant improvements in IL-6, NOS-1, and COX-2 among patients with T2DM.

Khout and associates investigated the effects of aerobic versus flexibility and resistance exercises on inflammatory markers along with other parameters in older adults. They suggested that aerobic exercise program of 3 days/week; 45 minutes/day for 10 months significantly improves serum inflammatory mediator atherosclerosis, T2DM, hypertension, and depression in adults of more than 64 years of age. This study also supported our result where improvements were demonstrated in IL-6,
NOS-1, and COX-2 among patients with T2DM, treated with SSAET program, routine medication and dietary plan. Itō and group conducted an animal study and concluded that regular running exercise has renal protective effects through NOS-1 upregulation and causes suppression of nicotineamine adenine dinucleotide phosphate-oxygenase and α-oxoaldehydes in the kidneys, additionally with decrease in hyperglycemia in Zucker diabetic fatty (ZDF) rats, undergone forced treadmill exercise for 8 weeks.18 These results also suggested significant improvement in NOS-1 level in patients with T2DM, treated with SSAET, routine medication and dietary plan.

Buford and team also suggested the upregulation of IL-6 and COX-2 was noted while downhill running of 45% at -17.5% of tread mill protocol of 10% maximal oxygen consumption was applied in 29 males between 18-35 years of age. The venous blood samples and biopsies of vastus lateralis muscles were taken 3 hours prior to exercise and after 24 hours of exercise.19 The present study also concluded significant improvements in IL-6 and COX-2 level in patients with T2DM, treated with SSAET, routine medication and dietary plan.

Markworth and associates conducted another study and suggested that a single acute bout of resistance exercise session equal to 80% of repetition maximum, produced lipid mediated inflammatory response in human and showed that pro-inflammatory signals are linked to the induction of biologically active inflammatory resolution program.20

CONCLUSION

SSAET along with routine medication and dietary plan has positive influence on IL-6, NOS-1, and COX-2 in patients with T2DM. SSAET program is recommended for the management of low grade inflammation associated with T2DM by targeting IL-6, NOS-1, and COX-2.

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REFERENCES


