ORIGINAL ARTICLE

Effect of obesity on serum IL-10 concentrations and messenger RNA expression in women with metabolic syndrome

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ABSTRACT

Objectives: In humans, the probable role of anti-inflammatory cytokines in obesity is unidentified. The objective of this work was to investigate serum IL-10 concentrations and the messenger RNA expression (mRNA) in peripheral blood of 30 obese women with metabolic syndrome (MS) and 20 lean healthy controls matching sex and age.

Methods:In this cross-sectional study, 50 Egyptian women (age <45 years) were included. Blood pressure, anthropometric measurements, lipid parameters, obesity indices and HOMA-IR were measured. The expression of IL-10 in blood was assessed by extraction of RNA followed by real time PCR analysis. The IL-10 level in serum was assessed using enzyme linked immunosorbent assay (ELISA).

Results: Women with MS had lower values of IL-10 and significant higher levels of blood pressure, lipid parameters, obesity measures and HOMA-IR compared to control women (all P <0.01). Similarly, mRNA expressions were down regulated in MS women compared to the control group (P = 0.003).

Conclusion: Our study demontstrated that obese women have low levels of IL-10, suggesting that this anti-inflammatory cytokine plays a crucial role in MS risk and obesity-related problems**.** Moreover, low mRNA expression suggests the contribution of genes to insulin-resistant states and the metabolic syndrome in obese women.

Keywords: Interleukin 10; metabolic syndrome; obesity; mRNA expression; cytokines.

N Z J Med Lab Sci 2022; 76(2): 69-73.

INTRODUCTION

Interleukin 10 (IL-10) has a crucial role in limiting inflammation and regulating the immune response. IL-10 represses inflammation via numerous mechanisms comprising supression of the of pro-inflammatory cytokines synthesis (1). Growing evidence has been found linking metabolic syndrome (MS) to IL-10, cardiovascular diseases, and obesity (2). It has been found that low circulating IL-10 is related to obesity in adults (3). Also IL-10 plays a protective part against insulin resistance development.

In young healthy adults, the concentration of IL-10 in plasma correlated positively with insulin sensitivity (4,5). IL-10 is secreted by T-cells, B-cells, macrophages and monocytes, this is under powerful genetic control, with heritability assessment as high as 75% (6). Genetic and environmental factors can share in the metabilic hazard. Genome wide association studies have found significant relations among MS and single nucleotide polymorphisms (SNP), predominantly located in genes encoding apolipoprotein synthesis and proteins that control the central nervous system hunger-satiety control (7). Gene variants of IL-10 have been related with type 2 diabetes mellitus risk (8); however, the association between gene expression of IL10 and its serum levels in Egyptian obese women with MS have not been previously investigated. The present study aimed to investigate serum IL-10 levels and the mRNA expression of IL-10 and the risk of MS in as sample of Egyptian obese women.

SUBJECTS AND METHODS

Study population

Thirty obese women between the ages of 25 and 35 years were recruited from the obesity clinic, National Research Centre. Exclusion criteria of the study group were pregnancy, systemic diseases (kidney, liver, heart, or any systemic diseases). The control group was twenty women with associated other endocrine disorder between the ages of 25 and 45 years. The study was approved by the Ethical Committee of the National Research Centre, Egypt (number = 16361), in accordance with the World Medical Association's Declaration of Helsinki. Written informed consent was obtained from all participants.

Anthropometric and clinical measures

Patients and controls were clinically examined and a full medical history was obtained. Anthropometric parameters were taken three times on the left side of the body and the mean value was used. Body weight, height, waist circumference (WC) and hip circumference (HC) were measured were measured as previously described (9,10). Body mass index (BMI) and waist hip ratio (WHR) were calculated. The anthropometric measurements and instruments followed the International Biological Program (10). Systolic and diastolic blood pressures (SBP and DBP) were measured three times with a standard mercury sphygmomanometer and appropriately sized adult cuffs on the right arm of each subject after a 10-minute rest in the sitting position, and the mean values were used for analysis. Metabolic syndrome (MS) and its individual components were assessed according to the National Cholesterol Education Program definition(11).

Measurement of glucose and lipids

Serum lipids (total cholesterol, high-density lipoprotein cholesterol (HDL-C) triglycerides (TG) and fasting plasma glucose were assessed using colorimetric enzymatic methods using a Hitachi auto-analyzer 704 (Roche Diagnostics, Switzerland). Low density lipoprotein cholesterol (LDL-C) was calculated according to a specific equation (LDL-C= Total cholesterol – Triglycerides/5+HDL-C). Serum insulin concentration was evaluated by a chemiluminescent immunoassay (Immulite 2000, Siemens, Germany. The insulin resistance was determined via the Homeostasis Model Insulin Resistance (HOMA-IR), calculated as the product of the fasting plasma insulin level (IU/mL) and the fasting plasma glucose level (mmol/L), divided by 22.5 (12).

Measurement of IL-10

The total concentrations of IL-10 in plasma samples were evaluated using an ELISA kit (R&D System, Inc., Minneapolis, MN), in accordance to the manufacturer's instructions.

Expression analysis of IL-10 RNA

RNA isolation and reverse transcription reaction

Total RNA from blood samples of control healthy persons (n=20) and from obese women with metabolic syndrome (n=30) was isolated by the standard TRIzol® Reagent extraction method (Invitrogen, Germany). After completion of the isolation procedures, RNA pellets were stored in DEPC treated water. To digest the potential DNA residues the pellet of isolated RNA was treated with RNAse-free DNAse kit (Invitrogen, Germany).

RNA aliquots were stored at -20°C or utilized immediately for reverse transcription.

First Strand cDNA Synthesis Kit (RevertAidTM, MBI Fermentas) was used to synthesize the cDNA copy from human samples via reverse transcription reaction (RT). A RT reaction programme of 25°C for 10 min, then one hour at 42°C then 5 min at 95°C was used to obtain the cDNA copy of human genome. Finally, tubes of reaction containing cDNA copy were collected on ice up to use for cDNA amplification.

Quantitative Real Time-PCR

SYBR® Premix Ex TaqTM kit (TaKaRa, Biotech. Co. Ltd.) was used to perform the qRT-PCR analyses using the synthesised cDNA copies from human samples. For each reaction a melting curve profile was conducted.

Statistical analysis

All obtained data were statistically analysed using SPSS16.0 software (SPSS Inc). To verify that the data of the present study was normally distributed the Kolmogorov–Smirnov test of normality followed by a Gaussian pattern was utilised. Normally distributed data in this study were exhibited as means ± SD. To examine the significant differences between the two studied groups, the data were analysed by Mann–Whitney U test or unpaired "t-test" as appropriate. All used tests were two-sided and considered statistically significant when $p < 0.05$.

RESULTS

The quantitative values of the target genes were normalised on the expression of the housekeeping gene (β-actin). The $2^{-\Delta\Delta CT}$ method was used to determine the quantitative values of the specific RNA to the b-actin gene (Table 1). The clinical characteristics of the studied population are summarised in Table 2. MS women showed significant higher obesity meaures, including BMI, WC, WHR and SBP as well as higher blood pressure levels compared with control healthy women (Table 2).

Table 3 shows the biochemical characteristics of the two groups. The results showed that MS women had significant decreased value of IL-10 levels and higher levels of serum lipids and HOMA-IR than the control group. Morover, MS women showed significant higher levels of TC, TG and LDL-C compared to control women.

The expression values of IL-10 gene ($2^{-\Delta Ct}$) were significantly decreased in MS patients (0.818) compared to controls (2.293), P=0.003. The relative expression levels of IL-10 RNA are demonstated in Figure 1, which exhibited that the expression levels in healthy control samples were significantly higher that those of MS patients.

Table 1. Primers sequence used for RT-qPCR

Gene	Primer sequences used for RT-qPCR	NCBI reference sequence
IL-10	F: GTT CTT TGG GGA GCC AAC AG	NM_000572.3
	R: GCT CCC TGG TTT CTC TTC CT	
β-actin	F: AGA GCT ATG AGC TGC CTG AC	Jing Z, *et al.* (13)
	R: AAT TGA ATG TAG TTT CAT GGA TG	

Table 2. Clinical characteristics.

Variables	Group	Mean±SD	*P*-value
Age (years)	Controls	33.90 ± 3.46	0.61
	MS	35.90 ± 4.45	
BMI (kg/m^2)	Controls	21.89 ± 2.08	0.01
	MS	33.28 ± 5.89	
WC (cm)	Controls	74.91 ± 9.039	0.05
	MS	100.07 ± 12.540	
WHR	Controls	0.76 ± 0.09	0.03
	MS	0.8463 ± 0.06	
SBP (mmHg)	Controls	93.89 ± 11.95	0.02
	MS	108.68 ± 15.64	
DBP (mmHg)	Controls	63.06 ± 5.18	0.02
	MS	71.76 ± 9.82	

BMI: body mass index; WC: waist circumference; WHR: waist to hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 3: The biochemical characteristics of the studied women.

Variables	Group	Mean±SD	*P*-value
IL-10 (pg/mL)	Controls	13.29 ± 3.61	0.01
	MS	10.82 ± 2.95	
Total cholesterol (mmol/L)	Controls	4.194 ± 1.68	0.01
	MS	6.21 ± 1.18	
Triglycerides (mmol/L)	Controls	0.80 ± 0.32	0.02
	MS	1.19 ± 0.47	
HDLc (mmol/L)	Controls	1.31 ± 0.33	0.04
	MS	1.13 ± 0.26	
LDLc (mmol/L)	Controls	2.56 ± 0.92	0.03
	MS	4.39 ± 1.13	
HOMA-IR	Controls	2.29 ± 1.09	0.01
	MS	7.19 ± 2.19	

HDLc = high density lipoprotein cholesterol, LDLc = low density lipoprotein cholesterol, HOMA-IR: homeostasis model assessment-insulin resistance.

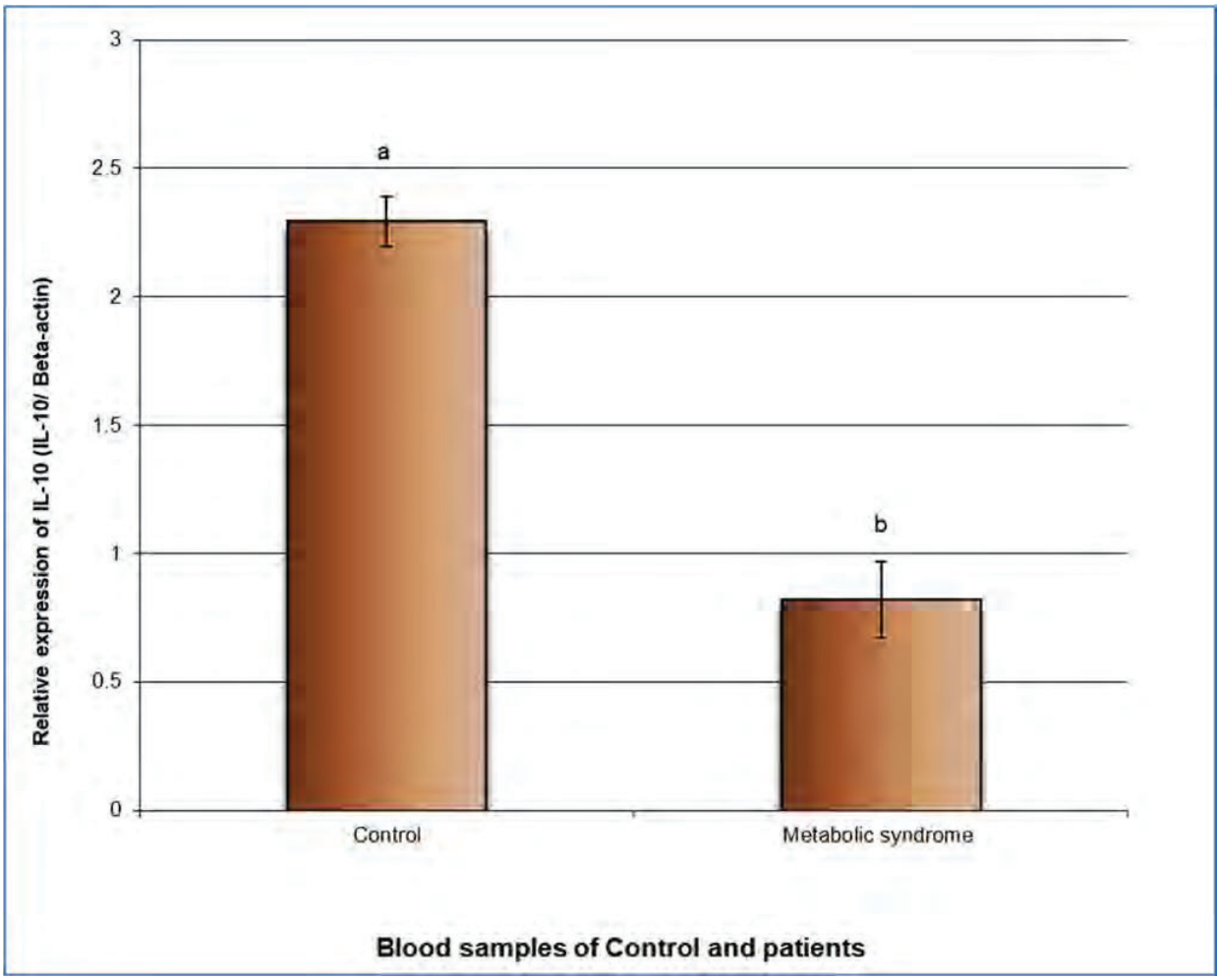

Figure 1. Expression alterations of IL-10 RNA in the control group (n=20) and the metabolic syndrome group (n=30). Data are presented as mean ± SD.

DISCUSSION

Various studies have shown increased inflammation in adipose tissue of obese subjects and disclosed its propable role in the progress of insulin resistance. The decreased production capacity of IL-10 (i.e., a pro-inflammatory response) is in association to type 2 diabetes and metabolic syndrome (6,14). MS is distinguished by related risk factors such as low high-density lipoprotein (HDL) levels, hypertriglyceridemia, impaired glucose tolerance, obesity (particularly visceral adiposity) and raised blood pressure. MS is an established clinical condition and a public health concern highly associated with the incidence of obesity, excessive caloric intake and sedentary lifestyle (15). Moreover , it has been shown that obesity and MS are accompanied with chronic low-grade systemic inflammation, particularly higher in women (16). Pro-inflammatory cytokines have been related to the development of type 2 diabetes and metabolic syndrome. The total cholesterol, triglycerides, LDL-C and HOMA-IR were significantly higher in the obese women in comparison to control. While HDL-C was diminished in the obese women compared to the control. This is interrelated with lipids accumulation in adipose tissue, secretion of lipoproteins, and increase in hepatic synthesis. Insulin resistance has been suggested as the leading cause for this cardiovascular and metabolic syndrome, despite that its molecular basis is not yet clear (17). Experimental studies in animals and humans have revealed that treatment with pro-inflammatory cytokines stimulates insulin resistance and hypertriglyceridemia (18-20). The eventuality of a significant correlation between HOMA and the studied variables was predominantly lower in male than female subjects. Such is the case for triglycerides, total cholesterol, HDL-cholesterol, interleukin-6, hs-CRP, adiponectin. and TNF-α (21,22).

Obesity is a multifactorial disease with epigenetic alterations (23). IL-10 is an anti-inflammatory cytokine with an imperative role in the immunological system regulation via inactivation of pro-inflammatory cytokines through the repression of macrophage function, with a consequentual crucial role in immunity. Gene expression of IL-10 in our study was deficient in the obese women compared to the control group. A previous study by Viesti *et al.* (24) did not delineate a difference in IL-10 expression among their groups. Other studies have reported an association between obesity and dysregulated expression of miRs in inflammatory adipocytes (25,26). On the basis of the present data, the low levels of IL-10 can be used as a treatment to ameliorate the obesity-related problems. It may be concluded that the correlation between the gene expression of IL-10 in individuals with obesity and MS in the Egyptian population extends the current understanding of the biological passages of obesity.

ACKNOWLEDGEMENTS

The authors wish to thank nthe National Research Centre for use of their facilities to carry out this work.

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REFERENCES

1. Brackenbury LS, Carr BV, Stamakati Z, et al. Identification of a cell population that produces alpha/beta interferon in vitro and in vivo in response to noncytopathic bovine viral diarrhae virus. *J Virol* 2005; 79(12) :7738–7744.
2. Nishida M, Moriyama T, Sugita Y, Yamauchi-Takihara K. Interleukin-10 associates with adiponectin predominantly in subjects with metabolic syndrome. *Circ J* 2007; 71(8): 1234–1238.
3. Bosutti A, Malaponte G, Zanetti M, et al. Calorie restriction modulates inactivity-induced changes in the inflammatory markers C-reactive protein and pentraxin-3. *J Clin Endocrinol Metab* 2008; 93(8): 3226–3229.
4. Ibrahim NA, Saleh NM, Koprulu F, Abdulrahim AH. Metabolic syndrome associated risk factors: Findings among female undergraduate university students. *Res J Pharm Technol* 2020; 13(12): 6093–6097.
5. Chang JS, Chang C-C, Chien E, et al. Association between interleukin 1β and interleukin 10 concentrations: a cross-sectional study in young adolescents in Taiwan. *BMC Pediatr* 2013; 13: 123.
6. van Exel E, Gussekloo J, de Craen AJ, et al. Low production capacity of interleukin-10 associates with the metabolic syndrome and type 2 diabetes: the Leiden 85-Plus Study. *Diabetes* 2002; 51(4): 1088–1092.
7. Fall T, Ingelsson E. Genome-wide association studies of obesity and metabolic syndrome. *Mol Cell Endocrinol* 2014; 382(1): 740–757.
8. Saxena M, Srivastava N, Banerjee M. Association of IL-6, TNF-α and IL-10 gene polymorphisms with type 2 diabetes mellitus. *Mol Biol Rep* 2013; 40(11): 6271–6279.
9. Zaki M, Kamal S, Ezzat W, et al. Serum apelin levels and metabolic risk markers in obese women. *J Genet Eng Biotechnol* 2017; 15(2): 423-429.
10. Hiernaux J, Tanner JM, Jarman S. Growth and physical studies. In: JS Weiner and JA Lourie (Ed). Human Biollogy: A Guide to Field Methods. Blackwell Scientific,1969.
11. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Circulation* 2005; 112(17): 2735–2752.
12. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28(7): 412–419.
13. Jing Z, Deng H, Ma J, et al. Expression of immunoglobulin G in human podocytes, and its role in cell viability and adhesion. *Int J Mol Med* 2018; 41(6): 3296–3306.

14. Kakar M, Chakarborty P, Behl T, et al. Insight into the role of Inflammation in progression of diabetes associated neuropathy. *Res J Pharm Technol* 2020; 13(11): 5477–5483.
15. Farinha JB, Steckling FM, Stefanello ST, et al. Response of oxidative stress and inflammatory biomarkers to a 12-week aerobic exercise training in women with metabolic syndrome. *Sports Med Open* 2015; 1(1): 19.
16. Esposito K, Pontillo A, Giugliano F, et al. Association of low interleukin-10 levels with the metabolic syndrome in obese women. *J Clin Endocrinol Metab* 2003; 88(3): 1055–1058.
17. Reaven GM. Banting lecture. Role of insulin resistance in human disease. *Diabetes* 1988; 37(12): 1595-1607.
18. McArdle MA, Finucane OM, Connaughton RM, et al. Mechanisms of obesity-induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies. *Front Endocrinol (Lausanne)* 2013; 4: 52.
19. Mohsen H, Shaden H, Faiza AL, Taghrid H. Correlation of serum leptin levels with insulin resistance in Syrian obese patients with type 2 diabetes mellitus. *Res J Pharm Technol* 2013; 6(10): 1149–1151.
20. Zaki M, Kamal S, Mona Abd Elmotaleb AH, et al. Comparative study of chemerin and inflammatory markers between obese with insulin resistance and lean healthy women. *Res J Pharm Technol* 2021; 14(7): 3647–3650.
21. Belhayara MI, Mellouk Z, Hamdaoui MS, et al. Relationship between the insulin resistance and circulating predictive biochemical markers in metabolic syndrome among young adults in western Algeria. *Diabetes Metab Syndr* 2019; 13(1): 504–509.
22. Chowdary RP, Praveen D, Aanandhi VM. A prospective study on incidence of dyslipidemia in diabetes mellitus. *Res J Pharm Technol* 2017; 10(2): 431–433.
23. Monalisa R. Role of leptin in obesity. R*es J Pharm Technol* 2015; 8(8): 1073–1076.
24. Viesti A Collares R, Salgado W Jr, Pretti da Cunha Tirapelli D, dos Santos JS.. The expression of LEP, LEPR, IGF1 and IL10 in obesity and the relationship with microRNAs. *PLoS One* 2014; 9(4): e93512.
25. Kiran S, Kumar V, Kumar S, et al. Adipocyte, Immune Cells, and miRNA Crosstalk: A Novel Regulator of Metabolic Dysfunction and Obesity. *Cells* 2021; 10(5): 1004.
26. Choi EW, Lee M, Song JW, et al. Fas mutation reduces obesity by increasing IL-4 and IL-10 expression and promoting white adipose tissue browning. *Sci Rep* 2020; 10(1): 12001